

Time to ban British boxing

Cockfighting and bear-baiting have been banned for several decades in most grown-up countries, but fist-fights between people are tragically still allowed.

BRITISH boxing killed another young man, a young Scot called James Murray (25), at Glasgow over the weekend. On this occasion, the tragedy was marred by a riot of Murray's supporters in the crowd, apparently angry that their man had been robbed of the British bantamweight title by his collapse less than half a minute before the fight was due to end. There have been suggestions that the riot delayed Murray's arrival at hospital, but there is no confirmation of that. It is unlikely that more speedy attention would have saved his life, from a ruptured vein in the brain. Meanwhile, everybody connected with the prizefight, from those who had arranged the occasion ("promoters") to Murray's manager and his opponent, has expressed regret.

They are hollow words. It is not as if the hazards of boxing are unknown. Earlier this year, an American fighter, Gerald McClellan, was similarly injured in a televised prizefight, carted off to a nearby hospital and helped to recover to a state of neurological incompetence, presumably permanent. This is the eleventh case of overt brain damage suffered in British boxing rings in less than a decade. Worldwide, there were more than 300 such cases between the 1945 and 1993, which figures do not include the numbers of ageing fighters who are left neurologically incompetent, or punchdrunk, after a necessarily short professional career in the boxing-ring. The surprise underlying the weekend's expressions of regret is entirely misplaced.

So why not use the law to ban spectacles in which pairs of people publicly set about hitting each other with their fists? The British Medical Association, to its credit, has been campaigning for legal restraints on boxing for more than a decade. The difficulty is that the prizefighting lobby has libertarian arguments on its side. And it is true that boxing is technically a voluntary activity, even if it is also one of the standard routes by which poor young men seek escape from their poverty. Moreover, a law that prevented young men from engaging in fist-fights with each other would be a great infringement of people's freedom to conduct their personal lives as they see fit. There may be hazards, but so there are in other dangerous pursuits, rock-climbing, for example. Why pick on prizefighting? the boxing promoters ask.

Sanctions

The legal ban on cockfighting, popular enough in seventeenth-century Britain and elsewhere, is an illustration of how the law can and should be used. The logical basis for banning cockfighting and other such spectacles is simple.

First, they entail cruelty to animals (which do not usually volunteer for their roles). Second, making a spectacle of cruelty to animals is degrading for those who pay to watch cockfights and, by extension, for the societies that allow them to take place. When laboratory scientists have to be licensed by governments to carry out procedures capable of causing pain to animals (if they are not anaesthetized), a lobby to bring back cockfighting would make very little headway. Turning the argument around, the legal sanctions that should be imposed on prizefighting rest exclusively on the argument that these spectacles are degrading for those who watch them, and for the societies that allow them to happen. Last Friday's events are a vivid proof of that.

Revenue

The boxing-lobby's rejoinder is that a ban on prizefighting spectacles would drive the business underground. So what? Boxing as it has become depends on the large fees that promoters earn from the sale of television rights, and on the substantial income from those who buy seats at boxing-matches, which can be secured only by public advertising and skilful marketing. Boxing driven underground would be unlikely to be able to recruit a steady stream of hopeful youngsters into a activity whose present attraction is the chance, however small, of fame and fortune. An illegal activity with small prizes would not be nearly as attractive. Legislation should not, of course, prevent young men from fighting each other with fists (or even boxing gloves) if that is their inclination and if they do so privately.

The boxing-lobby has a second line of defence: make boxing safer, the promoters will be saying in the days ahead. Under British rules, boxers must undergo a CAT-scan of the brain before they are allowed to fight; now the promoters are saying that an MRI scan might be more appropriate. Similarly, in the aftermath of the weekend's tragedy, they are letting it be known that there may be a case for a more thorough investigation of boxers' weights in the period before prizefights, so that people who have struggled to qualify for particular weight-limited fights do not enter the ring in a dehydrated condition. There has been no talk, as yet, of the use of weightless boxing-gloves or of making a blow to an opponent's head cause for disqualification. That is natural enough; the promoters know their business. Spectators are snared only by the promise that they will see one man inflict real, sometimes lethal, damage on his opponent. Emasculating boxing would make it more difficult to sell



tickets, which is further proof that it is degrading.

That is why the British government should listen carefully to the complaints there will be when the House of Commons reassembles this week, and plan to outlaw boxing as a spectacle. To the inevitable rejoinder that British boxers would go abroad if there were a ban, the government could afford to shrug its shoulders. There is no case for preventing people hazarding their lives as they wish; the issue is simply whether their activities presented as spectacles are compatible with the standards of an enlightened society. □

Prize for Pugwash

Pugwash is a deserved (and deserving) winner of the Nobel Peace Prize announced last week.

PUGWASH is not so much an organization as a club or, more strictly, a network of geographically based clubs of people in Britain, the United States, Russia and elsewhere. Although its formal purpose is the study of the issues arising from the use of modern weapons, and especially nuclear weapons, it has often seemed that its chief preoccupation has been raising the funds required to send members to meetings overseas. That should be a little easier now, with the unexpected arrival of the organization's \$500,000 share of the Nobel Peace Prize. But it is particularly pleasing that the other half of the prize goes personally to Professor Josef Rotblat, the founder of Pugwash and its senior figure for the past 40 years. He has been stalwart in keeping the organization alive when, on several occasions, it might have collapsed.

Pugwash, a creature of the Cold War, has come a long way in its 40 years. Although its original aim and practice was to find a way of talking to groups of Soviet (now mostly Russian) scientists about the dangers of nuclear war, it was often confused with the British movement for unilateral nuclear disarmament, founded at about the same time. But Pugwash became respectable when Western governments recognized that it had indeed captured the interest and attention of influential people on the Soviet side. The result was to win the interest of people as different (or as similar) as Henry Kissinger and Solly (Lord) Zuckerman, in their time advisers to the US and British governments. It is to Rotblat's personal credit that he never let Pugwash become their prisoner. More by good luck than good management, the organization also survived an attempt to broaden its scope, taking in the problems of development and environment; those who would have taken Pugwash in that direction in the early 1970s could not compete with nuclear weapons in the interest of what they had to say.

For most of this time, Pugwash has been more than a slightly chaotic organization. The perennial difficulty about funds is part of the explanation. So, too, has been the convention that the annual meetings would be held in private, but that a statement about each of them would be prepared by the governing council and proclaimed to the press. The result was inevitably a document so laden with statements that participants believed would carry weight with their gov-

ernments that they could only seem anodyne. But old Pugwash hands aver that they came to know their opposite numbers only in the all-night sessions in which the substance and the syntax of these documents were hammered out.

Does Pugwash deserve its Nobel prize? Certainly. But for a surprising reason. As the Cold War dragged on, the two then-superpowers (the United States and the Soviet Union) found other more professional ways of talking about nuclear strategy. Those are meetings from which have sprung the bilateral agreements on nuclear weapons now in force. Pugwash's influence upon those events has been that of a gadfly, a means of keeping officials on their toes. So what should Pugwash do next? Why not try to talk to China? That would be a worthwhile cause, with which the prize will help. But the lasting value of the prize will be to give substance to Rotblat's hope (see page 564) that it will persuade professionals in other fields that it is a public duty to spend time worrying about the implications of their work. □

Deficits cause pain

Global financial markets have made governments' fiscal deficits unsupportable.

WHY should the governments of all rich countries be worried about their budget deficits? In the United States, the Congress and the administration have just six weeks in which to decide between two plans to reduce the deficit to zero. In Britain next week, the Chancellor of the Exchequer will either say that the deficit is too high (at £25 billion a year) to allow room for tax cuts or he will offer marginal fiscal benefits and promise that there will be much more to come when the deficit is reduced. In France, the new prime minister, M. Alain Juppé, has already dispensed with one deficit-reducing finance minister and has seen the franc slide on the currency markets. And elsewhere in Europe, every government is trying to balance its books as the Maastricht Treaty requires as a condition of membership of the proposed common currency.

The explanation is what is called globalization. The international currency markets are now so large and so efficient that funds find their way to where they can be invested safely and profitably. That explains why the Deutschmark is the strong currency of Western Europe. Elsewhere, in the United States for example, real interest rates are historically higher than ever at about 4 per cent a year. That is a measure of the cost of funding the budget deficit and a sign that the market people believe that something will go wrong.

Fifteen years after President Ronald Reagan began playing fast and loose with the budget, the chickens are coming home to roost. In the United States and other places with chronic deficits, the only sure prospects of growth are in the cost of funding new debt and paying interest on what has already accumulated. Responsible governments are smart enough to read the signals. Others will find themselves in Queer Street. Either way, there are painful times ahead. □

Nuclear regulators to investigate 'contamination' incident at MIT

Washington. A post-doctoral fellow at the Massachusetts Institute of Technology (MIT) claims to be suffering from "pain all over [his] body" two months after an incident in the laboratory of Nobel laureate Susumu Tonegawa in which he appears to have ingested over 500 microcuries of the radioactive isotope phosphorus-32.

So far the incident, which took place on 14 August, has been kept quiet by MIT. But a team of inspectors from the Nuclear Radiation Commission (NRC) was expected to visit Tonegawa's laboratory this week to investigate it; the NRC says it was first notified of what happened by MIT on Monday, after the institute had been informed that *Nature* planned to report on it today.

A statement from MIT, which did not identify the researcher — Yuqing Li — or the laboratory at which the incident took place, said that it "had exposed the

researcher to an intake of about 579 microcuries of radioactive material," noting that this is "within the permissible one-time and annual limit of 600 microcuries".

The statement said that the researcher had been examined by the medical department, and was released to go home. He had been re-examined "a number of times", and "no physical health effects were noted". It added that the short half-life of phosphorus-32 meant that the amount of radioactive material in his body was now down to about 9 microcuries.

Six hundred microcuries of phosphorus-32 is equivalent to 5 rem, the dose at which MIT would be required by law to notify the NRC of the incident within 24 hours — a process which will normally make the incident public. One of the first tasks of an inspector, says an NRC official, would be to check what he called MIT's "claim" that the

dose was indeed 579 microcuries.

The dose estimate suggests that Li swallowed at least half the contents of a standard laboratory vial of phosphorus-32. That is at least ten times the amount used for a typical trace experiment, and enough to make accidental contamination extremely unlikely. MIT says its own investigations, conducted by its Radiation Protection Office and the MIT campus police, have been "unable to determine whether the radioactive material intake was deliberate or accidental".

Speaking from home, where he has been resting since the incident was discovered on 19 August, Li said that he had been in considerable physical pain since then. The Chinese researcher would not say if he was happy with the MIT investigation, or reveal any plans to seek redress. But sources close to Tonegawa's laboratory say that Li is considering legal action.

Tonegawa, who won the Nobel Prize in physiology or medicine in 1987 for his work in characterizing the T-cell receptor, declines to comment on the incident. He has a reputation for bringing an intensely competitive approach to his laboratory, which specializes in neurobiology as well as immunology.

MIT said its Radiation Protection Office "took control of radioactive substances in the laboratory" for nine days after the incident was discovered, and that "the laboratory has continued to operate every day". But scientists in the field say it would be difficult for the laboratory to operate effectively without the radioactive tracers, and it may face more disruption with the arrival of the NRC inspection team this week.

Victor Dricks, a spokesman for the NRC regional office at King of Prussia, Pennsylvania, which oversees MIT, said the NRC was uncertain whether business as usual could continue at the laboratory. "That's one of the things we're going to check out," he said.

NRC officials appear to be irritated that the institute failed to inform it about an incident that fell within a hairsbreadth of a legal reporting requirement. Until a few months ago, when a previous regulation was withdrawn, MIT would have been obliged to file a written report within 30 days for a dose one-quarter of that ingested by Li.

Colin Macilwain

Federal investigators are studying the circumstances under which a group of researchers at the National Cancer Institute in Bethesda, including a pregnant research fellow, appear to have been deliberately contaminated with the radioisotope P-32.

For details, see page 568.

NASA struggles to save Jupiter data

Washington. Engineers at the National Aeronautics and Space Administration's (NASA's) Jet Propulsion Laboratory in Pasadena, California, are working overtime in a frantic effort to understand an apparent problem with the Jupiter-bound Galileo spacecraft that could jeopardize the whole scientific return from the mission.

Telemetry data that were received last Wednesday (11 October) revealed that an onboard tape recorder, which will be used to store images and other data during Galileo's two-year tour of the Jupiter system, failed to stop after rewinding. Mission managers immediately put the recorder in "safe" mode and began studying the problem using an identical recorder on the ground.

The apparent failure followed a picture-taking sequence in which the spacecraft had taken three separate images of Jupiter, each using a different colour filter. The images were the first to be recorded on tape since a newly uploaded sequence of software commands came into operation on 9 October.

The failure of the tape recorder would be a serious blow to the Galileo mission, which is already suffering the loss of a

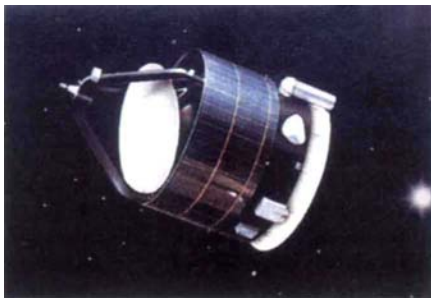
high-rate antenna that refused to unfurl properly four years ago. Project managers claim they can achieve almost three-quarters of Galileo's original science objectives by using a smaller antenna to send back digital pictures and other data at a much slower rate, namely 160 bits per second rather than 134,000 bits per second.

But that strategy depends heavily on storing data collected during close encounters with the planet and its moons, and playing it back later. The recorder, which has a capacity of 109 megabytes, is the only one on board the spacecraft.

The Galileo orbiter and a smaller atmospheric probe will both arrive at Jupiter on 7 December. The probe will plunge into the cloud tops, recording information on the pressure, temperature and composition of the atmosphere as it descends. At about the same time, the orbiter will fire retro-rockets that will slow it sufficiently to orbit around the planet.

At the beginning of the week, the precise nature of the recorder's failure remained unknown. Project engineers were planning to begin sending commands to the spacecraft by the end of the week, in the hope that this will shed further light on the problem.

Tony Reichhardt



Galileo: malfunctioning tape recorder could seriously limit success of planet mission.

Peace prize rewards physicist of conscience

London. Exactly 50 years after the atomic bomb was dropped on Nagasaki and Hiroshima, the Nobel Peace Prize for 1995 has been awarded to the first physicist to have resigned from the war-time Manhattan project in protest at the planned use of the bomb on Japan.

Joseph Rotblat, the Polish-born British physicist, says that he hopes the decision to award the prize jointly to himself and the Pugwash Conferences on Science and World Affairs, in which he has been active for the past 40 years, "will encourage scientists to think seriously about the social implications of their work, and devote a little of their time to address the dangers of science for society".

Having been for several decades at the forefront of international efforts to halt the arms race between the world's superpowers, Rotblat argues that, despite the ending of the Cold War, Pugwash's role remains as important as ever, pointing out, for example, the danger that nuclear weapons will be developed by "rogue nations".

Much of the almost US\$1 million in prize money — which will rescue Pugwash from a permanent state of semi-insolvency, due largely to its refusal to accept funding from governments — will therefore be devoted to its priority of a global treaty banning all nuclear weapons (and not merely their testing or stockpiling).

But the award, although widely welcomed, has been accompanied by its own controversy. This was stirred up by statements from members of the Nobel committee that the decision to honour the activity of a group dedicated to nuclear disarmament was an implicit criticism of France's nuclear tests in the South Pacific. No mention of the French activities was made in the formal citation. But Francis Sejersted, the chairman of the Nobel committee told a press conference in Sweden that the prize was a "specific message" to France.

Such statements have generated a fierce backlash in France, with several newspapers claiming that Pugwash — which, as Rotblat admits, has always maintained a low profile in its activities — might not have been awarded the prize without the intervention of President Chirac. This interpretation is

fiercely challenged by those who point to historical precedents, such as the award in 1985 to the International Physicians for the Prevention of Nuclear War, and the fact that, given its contribution to many recent arms limitation agreements, Pugwash had

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Path to peace: Rotblat (left) announces the outcome of first Pugwash conference in 1957, flanked by Cyrus Eaton (right).

apparently been hoping for several years for recognition of its efforts by the Nobel committee.

Rotblat is himself critical of the French activities, which he said had put the nuclear issue back on the international agenda. But he also admits to unease that Pugwash, which tends to avoid open criticism of any government because of its wish to maintain informal contacts, has been inadvertently caught up in the dispute. "I am sorry that the French tests have been brought in in this way," he says.

Rotblat's personal involvement with nuclear weapons took place during his work on the Manhattan Project at Los Alamos in the early 1940s, having been seconded from his position on the physics staff of Liverpool University (he left Poland in 1939 at the outbreak of the war).

He resigned from the project on hearing the news that Germany had dropped its efforts to develop a nuclear weapon — but that the project was to continue, with the new goal of dropping a bomb on Japan. "I joined the project because I knew that the Germans were doing the same thing; once they had stopped, I did not feel it right to continue," he says.

From that time on, Rotblat, while continuing to pursue an academic career — he was professor of physics at St Bartholomew's Hospital Medical College from 1950 to 1976 — devoted much of his energy to eliminating the weapons that the Manhattan project had created.

These efforts led directly to the creation of the Pugwash movement, named after the small town in Nova Scotia where the first meeting, financed by the industrialist Cyrus

Eaton, was held in 1957. Rotblat remained closely identified with the movement, acting as its secretary-general between 1957 and 1973, and chairman of British Pugwash from 1978 to 1988. Although now aged 86, he remains active in Pugwash affairs.

Unlike many better known organizations, Pugwash has preferred to operate behind the scenes rather than in public. The proceedings of meetings, to which government officials, academics and other scientific and policy experts from around the world are invited in a personal capacity, are held in private, with only a summary report issued at the end.

According to Rotblat and others, this strategy has paid off in laying the groundwork for a number of important international arms control agreements by dealing with the technical issues about how they are likely to be made to work. "The focus has been on the proposition that you have to get the science right, as well as pay attention to the interaction between the scientific and political aspects of problems," says John Holdren, professor of physics at the University of California at Berkeley, and chairman of Pugwash's executive committee.

The strategy of emphasizing the scientific dimension of problems has had the equally important spin-off of providing a channel of communication through which governments in conflict have been able to maintain a dialogue at times — such as the height of the Cold War — when more official channels have been blocked. Indeed, Rotblat claims that acting in this way has been Pugwash's greatest single achievement.

At the same time, this low public profile has made it difficult to spread the organization's more general message about the need for scientists to take responsibility for addressing the negative impacts of science on society, in particular to younger scientists. "There are different niches for different kinds of organization," Holdren says. "There have been some very effective mass membership organizations, but that is not the way that Pugwash tends to operate."

Furthermore, the thaw in East-West relations has removed much of the need for the type of back-room diplomacy which was Pugwash's forte. "It could certainly be argued that the influence of Pugwash has dropped off a bit," says one regular participant in its conferences.

But Rotblat and others insist that the continued threat of nuclear weapons — as well as more recent threats, such as the possibility for new generations of biological weapons based on the techniques of genetic engineering — mean that the need for Pugwash remains. "It is a different role than during the Cold War; but I do not think it is any less of a role," says Holdren.

David Dickson

Tighter ozone targets

London. The European Union has agreed to press for stricter controls on ozone-depleting substances when international talks on the Montreal Protocol take place in December.

Environment ministers decided to aim for the elimination of methyl bromide, used mainly as a soil fumigant, with a 50 per cent production cut by 2005, and the elimination of hydrochlorofluorocarbons by 2015. □

Ozone layer chemists represent Nobel 'first'

London. The Nobel prizes have often been criticized for failing to recognize the contributions of Earth and environmental scientists. That complaint may be heard less often following last week's awards of the chemistry prize to Paul Crutzen of the Max-Planck-Institute for Chemistry in Mainz, Germany, Mario Molina of the Massachusetts Institute of Technology and F. Sherwood Rowland of the University of California at Irvine, for their work in atmospheric chemistry.

The award citation highlights in particular their role in identifying the threat of the depletion of stratospheric ozone by anthropogenic compounds, and in elucidating the mechanisms by which this occurs.

Ozone is a trace constituent of the atmosphere, formed through photolysis of molecular oxygen by the ultraviolet component of sunlight. The highest concentrations are found in the stratosphere at an altitude of between 15 and 40 km; this 'ozone layer' absorbs most solar ultraviolet radiation. Identified in the early part of the century, the ozone layer was recognized in the 1930s to be a dynamic phenomenon, as ozone is constantly consumed and regenerated by natural processes.

Crutzen showed in 1970 that these processes are coupled to the biogeochemical cycling of trace gases at ground level. He demonstrated that the nitrogen oxides NO and NO₂, formed largely from nitrous oxide (N₂O) emitted by biota, catalyse the destruction of ozone. Ralph Cicerone of the University of California at Irvine says that this work "first brought ozone to the fore", showing that it could be affected by human activities. The following year, Harold Johnston suggested that nitrogen oxides released from supersonic aircraft might destroy stratospheric ozone.

More widespread concern followed the suggestion by Rowland and Molina in 1974 that chlorofluorocarbons (CFCs) used industrially as aerosol propellants, foam-blowing agents and refrigerator coolants might decompose in the atmosphere to produce chlorine free radicals, which could also catalyse ozone depletion.

As CFCs are chemically inert, their survival time in the atmosphere is relatively long, and they can therefore can find their way into the stratosphere. Here, ultraviolet light can split them into free-radical species, inducing the so-called chlorine catalytic cycle of ozone destruction.

In the United States, this warning led to a ban on the use of CFCs in aerosols. Elsewhere, however, little concern was expressed until a team from the British Antarctic Survey, led by Joseph Farman,

reported in 1985 that severe ozone depletion was occurring over Halley Bay in Antarctica.

The observation was a surprise not just because of its magnitude, but because it was occurring over the South Pole, whereas Rowland and Molina had predicted the largest effects in the tropics, where the intensity of sunlight is greatest.

Unravelling the reasons for polar ozone

surfaces of ice particles in polar stratospheric clouds. These reactions, in the dark of the polar winter, 'prime' the cloud particles for photochemical ozone destruction when sunlight returns in the spring.

In 1987, Molina and his co-workers helped to establish the basis of the chlorine catalytic cycle by highlighting the role of chlorine oxide (ClO) dimers. In collaboration with Neil Harris, Rowland showed that the effects of depletion could be seen at all latitudes.

Despite the award's emphasis on ozone, Crutzen's contribution to atmospheric chemistry has been more widespread. "No one has contributed as many ideas to the field," says Cicerone. Crutzen has worked extensively on the chemistry of the troposphere (at altitudes below 10 km), studying changes in tropospheric ozone levels (where it is a pollutant) and identifying the dangers of 'non-industrial pollution' from biomass burning.

Rowland says that atmospheric chemistry was for many years regarded by other chemists as "interesting but not chemistry". Both he and Molina regard the award as a sign that its scientific and social value is now

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Chemistry laureates: Crutzen (left), Rowland (centre) and Molina (right) helped to identify the threat of stratospheric ozone depletion.

depletion has involved a vast collaborative effort, in which all three recipients have remained active. By the 1990s it had become clear that the low temperatures above Antarctica are critical, and that ozone depletion here is not a result of homogeneous gas-phase chemistry but involves heterogeneous chemical reactions on the

Particle physics prize arrives at last

London. The main surprise of this year's Nobel Prize for Physics is not the names of the recipients, but the length of time that has elapsed before their recognition. The prize is to be shared by Martin L. Perl of Stanford University in California for his discovery of the tau lepton in the early 1970s, and Frederick Reines, of the University of California at Irvine, for the detection of the neutrino almost 20 years earlier.

Both discoveries significantly advanced understanding of the subatomic world and helped to establish the so-called Standard Model of particle physics, in which six leptons — consisting of the electron, the muon and the tau and three corresponding neutrinos — and six associated quarks are the elementary constituents of all matter.

The existence of the neutrino was predicted by Wolfgang Pauli in 1933. During the process of 'beta decay', an atomic nucleus emits an electron or positron, and simultaneously changes into the nucleus of a different element. Experiments in the early 1930s showed that energy was irretrievably lost in the process, suggesting that the principle of energy conservation might have to be abandoned for subatomic processes.

To avoid this drastic conclusion, Pauli proposed that the missing energy is carried

away by an uncharged and massless fundamental particle. It was a radical suggestion, because at that time only three subatomic particles were known — the electron, proton and neutron. Twenty-three years later, Reines and his collaborator Clyde L. Cowan (who died in 1974) announced the detection of the neutrino, calling it "the smallest bit of material reality ever conceived of by man" (see *Nature* 178, 446-449; 1956).

The tendency of neutrinos to interact with matter is exceedingly weak. To detect them, Reines and Cowan used a 400-litre tank of water and the predicted intense flux of neutrinos available from recently developed nuclear reactors, first in Hanford, Washington State, and later at Savannah River in South Carolina.

Henry Sobel, a colleague of Reines at Irvine, describes the experiment as "a very uncertain proposition, as the conventional wisdom at that time was that these particles were undetectable". But Reines and Cowan reported the detection of a few neutrinos per hour, fully in agreement with theoretical predictions of the time.

Reines has continued an active search for neutrinos. Recently, for example, he helped to direct the project that used an 8,000-tonne water Cerenkov detector in a salt ►

► mine near Cleveland, Ohio, to search for proton decay. The so-called IMB (Irvine-Michigan-Brookhaven) collaboration shared in the detection of a burst of neutrinos from supernova SN1987A, which strongly corroborated existing theories of the dynamics of stellar collapse.

While Reines and Cowan knew what they were looking for, Perl's discovery of the tau lepton was completely unexpected. "We started out trying to understand the relationship of the electron and the muon, but we just didn't make any progress," says Perl. "Finally we started to wonder if maybe there were more leptons; maybe there were a lot of them."

The discovery of the tau was made possible by the construction at the Stanford Linear Accelerator Center (SLAC) of a new accelerator called SPEAR, which Perl and colleagues began using in 1973. In the experiments, electrons and positrons collided at unprecedented energies, giving rise to a plethora of secondary particles.

Painstaking analysis was required to extract the signature of the tau from the raw data. "Our apparatus was primitive and incomplete, and we weren't very good at distinguishing leptons from pions," says Perl. Evidence for the new particle emerged within a couple of years, but Perl says that he and Gary Feldman, now professor of physics at Harvard University, "agonized over the data" before their findings were corroborated by other experiments.

Kurt Gottfried, of Cornell University, admits that when Perl said he had evidence that could only be explained by a new charged lepton, "people were very sceptical". But he says of Perl and Reines: "These are very excellent choices".

Sidney Drell, a colleague of Perl's for more than 30 years at SLAC, describes the award to Perl as a "superb choice"; the experiments were "brilliantly conceived and executed". Perl, he says, is "one of a kind", who likes to experiment off the beaten track.

Perl is currently attempting to detect free quarks. So far, the existence of quarks has been inferred from experiments on composite objects such as the proton. "Some people think this experiment is a waste of time," he says. "But a few have suggested that there may be free quarks left over as relics from the early universe".

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Reines: continuing the search for neutrinos.

French research centres told to make their patents pay

Paris. Elisabeth Dufourcq, France's secretary of state for research, has criticized public research organizations for not doing enough to patent their research results, holding up the aggressive patenting policies of the Atomic Energy Commission (CEA) as an example to follow.

In a bid to encourage researchers to place greater emphasis on patenting, Dufourcq said last week that the government is planning to submit legislation to the National Assembly during its 1995-96 session. This would include giving research teams one-quarter of the royalties arising from patents on their work, she said, adding that no ceiling would be imposed on such earnings.

"To submit a patent is to prepare the future, to plant a flag on a new land," said Dufourcq, who was speaking during a visit of the CEA's research centre at Saclay, near Paris. The CEA itself holds almost 1,400 patents, around 900 of which are derived from non-nuclear research. Last year the agency filed 158 patents, and its portfolio earned a substantial income.

In contrast, the patent portfolio of the Centre National de la Recherche Scientifique (CNRS) cost the agency as much as FF125 million in maintenance fees over the past decade. This situation, which critics have attributed in part to CNRS giving too large a share of royalties to licensees, has prompted the agency to review its policy on patenting and to prune back its

collection (see *Nature* 358; 531; 1992).

But the impact of the proposed measures is difficult to predict, particularly because the research organizations already provide individual researchers with a negotiated share of royalties on patents. It is also unclear, for example, whether the government intends to regulate the share of royalties demanded by research organizations from companies licensing their patents.

One senior official from the national biomedical research organization, INSERM, also contests the proposal that there should be no ceiling on researchers' earnings from royalties. Indeed, INSERM has been considering introducing just such a ceiling, he says, as it considers it unfair that some researchers should earn much more than others. The issue of such discrepancies is all the more sensitive, he adds, because INSERM's statutes forbid it to pay bonuses to staff who volunteer for administrative and other duties.

At the Institut Pasteur, whose income from patents tripled between 1988 and 1994, rising from 9 to 18 per cent of its FF860 million budget (see *Nature* 371, 277; 1994), researchers are not allowed to earn more than FF300,000 annually from royalties. Although the institute is a private organization, it hosts many CNRS and INSERM laboratories, and will need to review its policies when the decree is promulgated, says an official.

Declan Butler

Japan accelerates synchrotron plans

Tokyo. The world's most powerful synchrotron, Japan's SPring-8, is to be commissioned a year ahead of schedule, thanks to an extra ¥14.9 billion (US\$149 million) allocated to the project in a recent special supplementary budget designed to boost the flagging Japanese economy.

Project administrators at the Institute for Physical and Chemical Research (RIKEN), of the Science and Technology Agency (STA), claim that the accelerated schedule has been welcomed by the 900 or so Japanese researchers already recruited into the user's group to carry out experiments on the first 10 beam lines, now due to take place in late 1997. But they admit that the new schedule will make it difficult to recruit the foreign researchers needed to form a large international users' group in time for the commissioning of the 8-GeV synchrotron.

SPring-8 is being built by RIKEN in cooperation with the Japan Atomic Energy Research Institute (JAERI) and the ring will be administered by the Japan Synchro-

tron Radiation Research Institute (JASRI). User fees have yet to be decided, but a JASRI spokesman says use of the facility's beamlines will essentially be "free".

SPring-8 will eventually incorporate 61 beamlines, in addition to a 1-GeV linac and an 8-GeV synchrotron tunnel with a circumference of 400 metres. The first ten 'public beamlines', whose commissioning has been brought forward to February 1997, will be available for use by researchers from universities and national research institutes not directly associated with RIKEN or JAERI from October 1997.

The beams will be used for a wide variety of studies, including research on protein crystallography, soft X-ray spectroscopy of solids, high-energy inelastic scattering, nuclear resonant scattering, structural analysis of extremely dense states of matter under high pressure, physicochemical analysis, soft X-ray photochemistry, crystal structure analysis, and structural studies of expanded fluids at high temperatures.

Stephen Barker

Cuts raise questions over future of fusion

Washington & Paris. About 1,300 fusion researchers and support staff are destined to lose their jobs across the United States over the next year as fusion research enters a deep, painful and perhaps irreversible retrenchment. Despite claims elsewhere to the contrary, the cuts will inevitably cast a long shadow over global prospects for the use of fusion as a source of power.

The researchers include 260 laid off last week at the Princeton Plasma Physics Laboratory (PPPL) in New Jersey alone. They are victims of a so-called "continuing resolution" that fixes spending at a compromise rate, equivalent to around \$225 million a year for fusion against \$375 million last year, until the House of Representatives, the Senate and the Clinton administration agree a budget for the 1996 financial year, which began three weeks ago.

The cuts leave both the present and future US commitment to the International Thermonuclear Experimental Reactor (ITER) in the balance. Without knowing what its fusion budget will be, the US Department of Energy (DoE) has been working with ITER on how United States will fulfil its commitment to its partners — Russia, Europe and Japan — over the coming year on the reactor's design stage.

But this commitment is likely to be the minimum acceptable, as are any longer-term commitments to a project that many in the United States would like to see scaled-down. Although other ITER participants claim any unilateral move by the United States will not upset their own plans, a US withdrawal from the project will be a major blow.

According to sources in Congress, the US fusion budget is likely to end up close to the \$229 million proposed by the House of Representatives. There remains an outside chance, however, that it will win an extra \$56 million proposed by the Senate for PPPL (see *Nature* 376, 541; 1995).

At the lower level, according to Anne Davies, head of fusion at the energy department, what the United States can afford to do is not quite what ITER wants it to do. "It's a small difference," she says, "But it is going to be difficult to find it if we get the House budget."

With the main Tokamak Fusion Test Reactor (TFTR) now mothballed at Princeton, the DoE is understood to be preparing a new fusion energy plan for consideration at a meeting in November of its Fusion Energy Advisory Committee. It is expected to propose no new facilities, the closure of some existing ones, and to place the emphasis on the need for more basic research into plasma behaviour and materials.

As for ITER, its future after 1998, when the current design stage is scheduled for completion, has been questioned by a report

by a sub-panel of the Presidents' Council of Advisors on Science and Technology (PCAST). This proposed that ITER should be drastically scaled down from a cost of \$10 billion or more to just \$4 billion (see *Nature* 375, 713; 1995).

The PCAST panel called on the United States to seek "immediately" to renegotiate the international ITER agreement, so that

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Roger Ressmeyer, Starlight/SPL

Where next? Scientists check the tokamak fusion reactor at Princeton, which has laid off 260 staff.

the design stage could be adjusted for a smaller machine. "ITER is fine, provided that you also do research on advanced materials, advanced tokamaks and advanced steady state in parallel", says John Holdren, professor of physics at the University of California, Berkeley, and chairman of the panel. "It is crucial, even at a time of limited resources, to pursue those other lines of research."

But this proposal was roundly rejected by the ITER council in July. Robert Aymar, the director of ITER, claims there is no need to renegotiate the current agreement, and is confident that the decision to build ITER, "either alone or together", will be taken when that agreement expires in 1998.

"The United States thinks its view is the world view, but it's only the US view," says Aymar. He claims that PCAST has difficulty accepting that an international project can be more important than a domestic programme, and needs to accept the United States "is not the leader in fusion research".

If the United States decides to make its continued participation in ITER conditional on a radical reduction in size, the other part-

ners will be left with a difficult choice. ITER's *raison d'être* as a key step towards fusion energy would be diminished. But a decision to proceed without the United States would require the other partners to reaffirm their political support for fusion, which some might be reluctant to do in the light of the US withdrawal.

Certainly ITER has little incentive at present to take up PCAST's proposal to reduce its scale. The uncertainty of the US budget process means that, even with this concession, the other ITER partners would have no guarantee of continued US participation.

Moreover, the United States seems unlikely to renege on its current commitments to ITER, which continue until 1998. John Gibbons, President Bill Clinton's science adviser, said this month in a letter to Evgeni Velikhov, the chairman of the ITER council, that "at this point" the US administration did not wish to renegotiate the existing agreement.

In the short term, Europe's commitment also appears secure. Under an agreement reached in December 1993 as part of the Fourth Framework Programme, the European Commission has promised to provide ECU200 million (US\$244 million) a year for fusion research until 1999.

But in Europe, too, doubts about medium-term prospects for fusion are not far below the surface. In particular, the European Parliament, which has long contested fusion's claims to offer cheap, safe and limitless energy, could well oppose maintaining or increasing funding for fusion after 1999, particularly after last year's nomination of Detlev Samland (Socialist, Germany), a critic of fusion research, as head of the parliament's budget committee.

Meanwhile, support for fusion research remains strong in Japan, which has limited non-nuclear resources. During the summer, Keidanren, the powerful Japanese Federation of Economic Organizations, which is made up of the chairmen of major Japanese industrial groups, called for "nationwide support" for ITER, and launched a campaign for the reactor to be sited in Japan.

Colin Macilwain & Declan Butler

Tritium accelerator project gets the green light

Washington. Hazel O'Leary, the US Secretary of Energy, has formally announced her support for plans to build a proton accelerator to create tritium for US nuclear weapons, confirming earlier indications that she had rejected alternative proposals to construct a nuclear reactor for the purpose (see *Nature* 376, 201; 1995).

O'Leary intends to spend \$280 million over the next three years on research and development on the accelerator option, and a further \$46 million investigating the third

alternative, namely converting an existing nuclear power plant to produce the tritium.

The accelerator R&D programme will take place at Los Alamos National Laboratory in New Mexico, while the production accelerator will eventually be built at Savannah River, South Carolina.

Vic Reis, assistant secretary of energy with responsibility for nuclear weapons, appears to have ruled out the "dual-use" of the proposed accelerator by military and civil scientists.

C. M.

FBI investigates radiation exposure claims

Washington. The Federal Bureau of Investigation (FBI) has launched a criminal investigation into the circumstances under which 27 researchers at the National Cancer Institute in Bethesda, Maryland, including a pregnant research fellow, were contaminated — apparently deliberately — with the radioisotope phosphorus-32.

The US Nuclear Regulatory Commission (NRC) is simultaneously investigating complaints from the fellow, Maryann Wenling Ma, and her husband and laboratory partner, Bill Wenling Zheng, that lax safety procedures at the National Institutes of Health (NIH) contributed to the contamination incident. Lynne Bernabei, one of Ma's three lawyers, says Ma plans to sue NIH for the emotional distress she believes the incident has caused her.

In late June and early July, 27 researchers at the NCI's Laboratory of Molecular Pharmacology, were found to have abnormally high concentrations of the radioisotope, after drinking from a bottled water dispenser. Ma claims to have received the highest dose by eating leftover food spiked with the radioisotope. Ma, who is on a visiting fellowship from China — she was chosen in 1994 as one of the top 100 outstanding Chi-

nese young scientists of the year — was 17 weeks pregnant at the time.

The FBI has yet to issue a formal statement. The NIH has acknowledged that radiation contamination took place. But deputy director Ruth Kirchstein says that the agency will put up a vigorous defence against any charges of negligence.

"According to leading experts in the treatment of exposures to radioactivity, there is no reason to believe that Ma has been injured or her pregnancy compromised by the amount of radioactivity to which she was exposed," says Kirchstein.

Ma alleges in her complaint to the NRC that unauthorized access to radioisotopes was not impossible. She claims radioactive materials were rarely locked where she worked. But the agency refuses to confirm or deny Ma's claims that food was deliberately spiked. In addition, it disputes Ma's assertion that she received a higher dose than other researchers, and believes Ma's estimates for contamination are inflated.

Ma claims she received close to 1,000 microcuries, 16-times the yearly limit recommended by the NRC for pregnant women. But the NIH says it stands by its calculations that each of the contaminated researchers

received between 300 and 600 microcuries — below the annual occupational limit for radiation exposure. Tests conducted by the NRC showed she received between 740 and 820 microcuries. Ma suggests that the NIH's urine and blood collection procedures diluted the samples.

In her formal complaint to the NRC, Ma also suggests that her supervisor, John Weinstein, a researcher at NIH for the past two decades, tried to persuade her to have an abortion to avoid disrupting a project involving Ma and her husband. Ma adds, he "tried to convince us that since our experiments had involved radiation, the baby we expected would not be safe".

Ma also says that Weinstein convinced her not to tell the NIH Radiation Safety Branch that she was pregnant. The couple were deciding whether to inform the radiation branch of her condition when a routine check five days later revealed she had become contaminated, she says.

Weinstein has not been available for comment. But his lawyer, Morris Topf, said Weinstein "categorically denies" suggestions that he urged Ma to abort the baby, describing such charges as "preposterous, slanderous and incomprehensible". **Adrianne Appel**

Spanish research ambitions may face spending squeeze

Munich. Spain's science budget would increase by more than a quarter in the next four years under the third national research plan, approved last month by the government. The plan also proposes a shift in public spending from fundamental to applied research. But, given the pressures on public spending, many feel that its ambitious goals are unlikely to be met.

Following Spain's first national plan in 1988, money spent on research has grown on average by 10 per cent a year, and the number of researchers has increased from 26,000 to 42,000. The government has since backed away from such a high rate of increase; this year, for example, the science budget grew by only 6.4 per cent. Nevertheless, the new plan, which runs from 1996 to 1999, envisages a 28-per cent increase in state spending.

The research plan aims to raise national spending on research and development (R&D) from 0.84 per cent of gross national product to 1 per cent. It admits, however, that reaching this target would require a 70 per cent increase in industry's investment, raising the proportion of total R&D costs carried by industry from 46 per cent to 53 per cent, the European average.

Enric Banda, the Spanish research minister, claims that the huge increase in state financing of research over the past eight years "has allowed our science to mature, so we are now ready to ask research to help

industrial interests". Hence the increased emphasis on applied research. "All European countries have done this, and we have instituted only a slight and controlled change," he says.

The change will include introducing more national research programmes on applied themes, such as telecommunications, biotechnology and environmental technologies, coordinated between ministries, as well as programmes that require both academic and industrial partners. It was drawn up after detailed consultation with 500 experts from both the academic and industrial sectors,

and has already found considerable support.

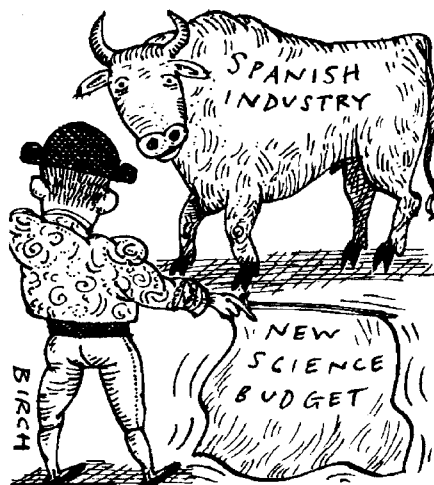
Pere Puigdomènech, for example, director of the Molecular Genetics Institute of the Consejo Superior de Investigaciones Científicas (CSIC) in Barcelona, says that "it may be a good thing to have some more direction". But he adds that scientists are worried that Spanish industry, which does not have a strong research tradition, may not be able to participate as fully as the ministry hopes in the planned developments.

The government is also suffering from economic difficulties. Despite ambitious talk of growing budgets, the general Spanish budget proposal for 1996 does not meet the full increase in science funding suggested in the national plan.

Moreover, public funding for CSIC, which runs 87 research institutes but does not provide project money (which is allocated competitively from the research programmes of the national plan), is marked down for a three per cent cut under the 1996 Spanish budget proposal. This would prevent CSIC from carrying out any new building next year.

However, the 1996 budget appears unlikely to be passed, and the socialist government may be forced to resign as a result. Indeed, few scientists believe that the new government, whatever its political complexion, will be prepared to fully fund the national plan.

Alison Abbott



Inquiry at Harvard prompts research paper corrections

San Diego. Three prominent physicians from Harvard Medical School working at the Children's Hospital in Boston have been forced to publish an extensive correction in connection with federally funded research on a treatment for rare, life-threatening birthmarks in infants.

The correction has been published in the *New England Journal of Medicine (NEJM)* after a lengthy investigation by Harvard into the conduct of the physicians, R. Alan B. Ezekowitz, John B. Mulliken and Judah Folkman. The latter is internationally renowned for his discoveries on angiogenesis, the process by which blood vessels grow.

It follows a briefer correction, published unilaterally last year by the three when the investigation was still under way, which included corrected data about some of the patients involved in the study, but without any accompanying comment — or even identification of the names of the authors.

The investigation concerned an article published in the *NEJM* (326, 1456–63; 1992) on the effectiveness of interferon alfa-2a in reducing large hemangiomas, growths that can occasionally block an infant's airway, affect its vision or compromise a vital organ.

The article, based on observations of 20 cases, claimed that the therapy had had a substantial effect. But Harvard officials say that after it was published, other physicians who had referred the children to the team complained about various inaccuracies. According to David Nathan, physician-in-chief at Children's Hospital, this prompted Harvard to set up an ad hoc committee to investigate possible misconduct, the first such probe at the 325-bed institution.

The committee found that there was no misconduct or fraud, says Nathan. But it did find many errors needing correction. These included the fact that some hemangiomas were reported to have regressed more than they did; that the lesions were not measured, as the article suggested; that some patients were not treated for the period reported; that prior therapies used on some patients were not listed correctly; and that monthly blood tests were not taken on all patients as reported, with the result that no conclusion could be reached on drug toxicity.

The results of Harvard's investigation were sent to the National Institutes of Health (NIH)'s Office of Research Integrity (ORI), which, according to Nathan, accepted the university's findings. But it appears that neither the National Cancer Institute (NCI), nor the National Center for Research Resources — the NIH agencies whose grants were cited as funding the research — was notified of the Harvard investigation or of any corrective actions.

Collette Freeman, the NCI grant officer, described the situation as "extraordinary" and "bizarre" when told of the correction.

Harvard officials refuse to comment on the case, or to say whether any sanctions were issued against the researchers. Nor were any of the three physicians prepared to comment. But in their correction, published on 31 August, they wrote: "We are embarrassed by our errors and regret them. We apologize to the readers for any misunderstanding and thank our colleagues for their assistance in correcting our report."

Nathan called the interferon research "a very important paper" by "distinguished investigators". But, he claims, the researchers "got very busy" and "just slapped this paper together". He accepts that almost all the errors in the eight-page article tend to improve the apparent success of the therapy. "That is how the original complaint was made," says Nathan. "It is curious that it turned out that way."

The lengthy investigation appears to have been marked by contention, defiance by the researchers and anger. Nathan says that when the Harvard review started, the three "became very irritable", submitting material for correction to the journal against his advice. "The first correction wasn't certified by Harvard," said Nathan. "I felt it was wiser to be patient, collect all the errors and deal with them in one fell swoop," he says, adding that the medical school feels strongly that corrections have to be certified by the institution.

Jerome P. Kassirer, who took over as editor-in-chief at the *NEJM* after the first correction was published, says that the Harvard researchers submitted "material that was very confusing" for this correction. Earlier this year, he says, the three physicians submitted further material, approved by Harvard, but this was little easier to interpret. "The editors had a great deal of trouble figuring out what they were driving at."

Despite the errors, Nathan said the conclusions of the study remain valid, and that interferon remains the preferred therapy for such hemangioma cases. But some physicians feel a random, prospective study is still required to back up this conclusion, as the lesions are known to regress on their own as children age.

"I have to express reservations about any non-randomized study, especially one in which it now appears that the data was brushed up," says Ajay J. Vora, a paediatric haematologist at Children's Hospital in Sheffield, in the United Kingdom, who originally questioned the interferon study in a letter to the *NEJM* in October 1992.

Rex Dalton

US plays hard to get as Unesco continues to woo support

Paris. Even hardened spectators of the long-running saga over whether the United States will rejoin the United Nations Educational Cultural and Scientific Organization (Unesco) — which it left in 1984, complaining of chronic mismanagement and anti-Western bias — are confessing bemusement at the latest chapter.

Speaking at a press lunch in Paris last week, Federico Mayor, the director general of the UN agency, claimed that President Bill Clinton had written to him in January saying the United States was prepared to

rejoin, as it felt the agency had been sufficiently reformed, and that the only problem was that it could not raise the \$65 million needed to pay its annual membership.

But the official US position appears less clear-cut. Recommendations from a government task force in 1993 that the United States should rejoin has never been formally endorsed by the administration. Moreover, says one State Department official, the election of a Republican majority in Congress, keen to cut payments to international agencies, means that the chances of rejoining Unesco in the near future are virtually zero.

The official also gives a somewhat different account of the events described by Mayor. He says that Mayor understood that the domestic US political scene ruled out the United States rejoining Unesco soon, but was concerned that the other member states might conclude that "there must be something wrong with Unesco if the US is staying out".

Mayor therefore asked the US administration to help to boost Unesco's credibility by issuing a statement saying it was satisfied with the reforms at the agency, according to the official.

One formula being considered is that used by Clinton in his letter in which he "applauded the substantial progress" being made by Unesco in addressing the problems that led to US withdrawal.

This letter was not in fact addressed to Mayor, as the latter claimed, but to Cyrus Vance, co-chairman of the national council of the US United Nations Association, who had earlier written to Clinton asking him to include funding for Unesco membership in the 1996 budget.

Declan Butler

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Thomas Haley/Rex Features

Mayor: wants backing from the United States.

Anthropology and race

SIR — While the race issues you raise are indeed serious, they miss the nub of the problem. And that problem, I believe, is exacerbated rather than solved in your leading article¹. The problem is the deprofessionalization of physical anthropology as a scientific discipline, and the substitution of folk wisdom for informed contemporary professional judgements. This is precisely what Dr Roger Bannister, the focus of your leading article, has done. And yet you did not cite a modern work in the field of physical anthropology in response. In fact the field is considerably different from your representation of it. The idea that the human species is naturally partitioned into a few large groups is antiquated by several decades, having been replaced by the recognition that (1) human biological variation is simply geographically patterned (people are similar to those nearby and different from those far away); and (2) large-scale racial clusters are cultural constructs, arbitrarily ignoring considerable diversity in order to contrast extremes².

Diverse human groups do of course sometimes consistently perform differently in certain arenas. The folk interpretation of this is that it reflects constitutional differences among them. On the other hand, anthropologists in this century have demonstrated that the human mind and the human form are possessed of considerable developmental malleability, through studies of similar peoples in different circumstances and different peoples in similar circumstances³. Although there is no way at present to study the genetics of ability or potential, what we do know of modern physical anthropology strongly undermines the assumption that consistent differences in observed performance are invariably or even primarily caused by hereditary or constitutional differences. Some may be, but a demonstration in any particular case demands considerably greater standards of validation than simply the observation of a consistent difference in performance. Certainly social history — the varied successes of different minorities at different times in different endeavours — suggests that there are no great differences in human potential at the level of populations. The “cognitive elite” described in *The Bell Curve* — Ashkenazi Jewry — was actually considered to have such corrupt “germ plasm” in the 1920s that legislation was deemed necessary to restrict their further immigration into the United States. Apparently they’ve improved since then. To the extent that individual differences may exist, we lack a social mechanism for evaluating and cultivating everyone’s diverse potentials. What we do know is that it simply is neither scientifically valid

nor admirably humanitarian to evaluate them by recourse to folk prejudices about the potentials of their groups.

The tragedy of the Human Genome Diversity Project (HGDP) is that it shows a different face of the same general problem. Anthropologists have been studying genetics of non-Western people for decades, but never with such great fanfare. Unfortunately, they were not among the project’s original designers; the HGDP was formulated initially by well-intentioned molecular population geneticists, with largely a folk knowledge of anthropology^{4–6}. Like Bannister, they tacitly assumed that the relevant anthropological issues were largely intuitive, and that modern anthropology itself could be largely ignored. They are now having to cope with the consequences of that assumption.

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■ see also Book Review on p. 589.

Climate change

SIR — In his article “Climate change: a successful prediction?”, Tom M. L. Wigley (*Nature* **376**, 463–464; 1995) comments on the study of Mitchell *et al.* (*Nature* **376**, 501–504; 1995) and undertakes a balanced review of recent findings emerging from climate modelling efforts.

While there is considerable work done on global-scale assessments of the phenomenon of climate change and its impact, confidence in regional-scale assessments based on global climate models is still low. Mitchell makes the point that “the improvement in simulation of specific regions is equivocal”. In fact, much of the pioneering work being carried out at centres of excellence such as the Hadley Centre in the United Kingdom, the Max Planck Institute in Germany and the Oak Ridge National Laboratories in the United States appears to lose sight of the need to refocus climate studies by making a transition from global generalities to regional/country specifics.

Leading atmospheric scientists in India are of the opinion that very few of the nuances associated with monsoons have been recognized or incorporated in models. And, unfortunately, despite the century-old tradition of meteorological forecasting, climate modelling in India is

still at an early stage and lacks the financial and computational resources to fill this crucial gap in international research.

South Asia, with its region-specific, agro-climatic zones, food security problems, rising population and economic growth — it must be squarely recognized — presents an immense challenge and opportunity to further and promote international scientific cooperation in this frontier field. This alone will help in assessing regional and sub-regional effects and provide an appropriate input to policy-making. The grim alternative is to negotiate climate change against the backdrop of an asymmetry of scientific information and political influence.

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Exploited authors

SIR — I share Keith Frayn’s view (*Nature* **375**, 100; 1995) of the attitude of publishers towards authors. About a year ago, I was invited to contribute a chapter for a new volume of the *Encyclopedia of Fluid Mechanics*, to be published by Gulf Publishing Company in the United States. I was given a submission date of 15 February 1995 and the editor sent me all the relevant documents.

In order to meet the deadline and to keep up with my own work, I paid a typist and a draughtsman from my own pocket. I also spent a lot of time seeking permission to reproduce material from other publications. No fee was offered but I was told I would receive a complimentary copy of the volume concerned. As I had been asked to write the chapter, I assumed that my expenses would be reimbursed.

The editor stopped communicating with me when the chapter was almost ready. In spite of my letters and fax messages (which are quite expensive in India), I have heard nothing more since then from either the editor or the publisher.

Imagine my surprise, therefore, when, in June 1995, the same editor asked me to contribute a chapter on a different subject to an *Encyclopedia of Polymer Processing Technology*, to be published this time by Marcel Dekker. What should I say to the editor? If I agree, will I hear from him again this time? Where do I stand with regard to the earlier contract?

I spent a great deal of time and some money preparing the earlier chapter. I can earn more money, but I cannot replace the lost time. Editors and publishers like those I have mentioned are simply exploiting willing authors.

Anil Kumar

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How could EU research be improved?

John Maddox*

My purpose is not to belittle the work the European Commission already does in support of European research, but to argue that there is a need for a more explicit science policy in Brussels — a greater clarity of purpose.

THESE days, it is difficult for somebody from Britain to suggest that the European Commission falls short of perfection in any respect without being regarded as one of the band of Eurosceptics who flourish across La Manche. That's especially true when there are repeated disappointments for well-wishers of the European cause; there was another last weekend, at Majorca, when the heads of state seemed to have spent more time on recrimination than on constructive talk about next year's inter-governmental conference. There is no defence against that kind of slur except a forthright declaration on my part.

Here it is. My view is that the greatest disappointment in Britain's relationship with the rest of Europe came in the summer of 1940, when Winston Churchill flew to France to offer "indissoluble union" to the defeated government, and was turned down. I was a schoolboy at the time. I vividly remember the keen sense of loneliness my pals and I felt at what seemed a needless rejection of a quixotic but seriously intended gesture. The point is that Churchill would have carried the House of Commons with him. Where, I wonder, would Europe be now if he had had his way? As it was, my pals and I became less diligent at learning how to conjugate the irregular verbs.

The rest of the story is all too well known. Post-war Britain was a curious blend of desolation and optimism, hardly recognizable anywhere today. The new government had a mandate for a series of brave social experiments, of which the splendid National Health Service is almost the sole survivor. Britain was also fighting a guerrilla war in what is now Malaysia, was preoccupied with places such as Aden, dispensing with an empire (in India), settling the affairs of what the French call the Proche-Orient, building nuclear weapons... and wondering whether it would ever be possible to dispense with the rationing of food and clothing.

Britain stood aside from the talks initiated by M. Maurice Schumann leading to the Treaty of Rome — and then rushed to sign the treaty that established Euratom. Especially after the Anglo-French adventure at Suez, I suppose it seemed natural to join whatever clubs would have us. It's interesting that all that was happening at about the time that Watson and Crick described what

proves to be the correct structure of DNA.

The rest of the tale is familiar, even boring. In 1963, General Charles de Gaulle said "No", but Edward Heath, the Conservative prime minister, successfully negotiated membership of what is now the European Union (EU). Then Harold Wilson, who succeeded Heath as prime minister in 1972, cynically called a referendum on European membership *not* out of deference to public opinion, but to still the grumbling of the left wing of his own party. Like many others, I left the Labour Party at that stage. But at no stage did either Heath or Wilson tell the British people that membership of the Union was an opportunity. "It will make no difference!" is what they said.

This is a long-winded declaration, but it's necessary to what follows. My own hopes for the Union are that it will indeed be a congenial place: healthy and wealthy — wealthy enough to be compassionate towards the disadvantaged within Europe and from elsewhere. As a Welshman, I'm also anxious that Europe should remain as culturally diverse as it has always been.

Those of us in science would also like to enjoy some of the fun there must have been when all our heroes, from Copernicus onwards, were telling us what the world is really like. That's a reasonable goal, but no longer a national goal.

Success

So how could the European Commission do better? Let's face it, the Commission is a stop-gap institution, an entity that will either disappear if the Union falls apart or be transmogrified into a civil service if "ever closer integration" is indeed what happens in Europe. Meanwhile, on research, it's only proper to say that the Commission's done some excellent things. Here are some of them.

Take the genome of bakers' yeast, or *Saccharomyces cerevisiae*, for example. Some years ago, the various Human Genome Projects recommended that yeast would be a good proving-ground for the techniques needed for telling the nucleotide sequence of the whole human genome; it's roughly a tenth as long, and there are only 16, not 23, independent chromosomes. And it's remarkable how often the genes of yeast are similar to those of higher organisms such as people.

Imaginatively, the Commission took this project under its wing. The result has been a great success. Three or four — I've lost

count — of the chromosomes have been sequenced in total already. The others will not be far behind.

There are three things to say about the project. First, it's worked, despite the complexity of having 30 or 40 scientists throughout Europe working on different parts of the same yeast chromosome. That's a triumph. Second, it's been an effective way of spreading the modern techniques of genetic analysis throughout Europe. In places as far apart as Barcelona and Manchester, there are people whose confidence as scientists has been enormously enlarged by participation in this and related programmes. Third, the scientific value of the result, academic though it may still be, will be of great value to the understanding of the human genome. The project is both excellent science and a model for collaboration between dispersed centres.

That's not all. Some years ago, Europe's radioastronomers were unable to raise from their national research councils the funds they needed for comparing signals from several different radiotelescopes pointing at the same radiosource at the same time. That's the technique called Very-Long-Baseline Interferometry, or VLBI. It's important not just in radio-astronomy, but as a means of telling how fast the Atlantic's getting wider. (It's about 3–5 cm a year.) Eventually, after a lot of prodding from the likes of us, the Commission made the modest grant that allowed the work to go ahead at Dwingeloo in the Netherlands.

Others will have their own opinions of what the Commission has done and is still doing well. For my part, I liked the old Science programme, the scheme for making small grants to support collaboration between researchers in two or more European countries. It didn't cost very much, but the Commission was always saying what a good scheme it was. So were the grant recipients. Between them, they persuaded me. And then the scheme was stopped, suddenly, in 1992. I'm still not clear what the reasons were, but they may have been more to do with politics than with rational management.

That brings me to the first of the questions I want to raise about the way in which the Commission functions: can we please have more continuity, at least where basic science is concerned? National research councils know that it often takes years for the administration of a new scheme to

*After-dinner talk, OST/SPRU/Nature conference Paris 28–29 September 1995.

become smooth, and familiar to those who will benefit from it most. It's crucial that the decisions should also be made by people who are identified working scientists, not officials; otherwise the decisions cannot routinely command respect.

I may be exaggerating, and I may be quite unfair, but I have the impression that each new commissioner for research brings a new agenda. The Human Mobility Programme appeared like that, like a rabbit out of a hat, with the arrival of Signor Filippo Pandolfi. Now we're told that Mme Edith Cresson is in love with the electric car. Let's hope that doesn't mean that the Human Mobility Programme will be scrapped in favour of vehicle research, although I suppose that in that case it would be possible to keep the name.

Deliberation

Can we please also have more deliberation? I confess that it's several years ago now, perhaps it was 1990, when an ex-student telephoned to explain that he was applying for an Esprit grant, and did I happen to know of a company in France or Germany, or preferably in both countries, that would be interested in collaboration. He said he'd be very grateful for a swift reply. "The applications are due next Friday." I gather that there was a great deal of last-minute fuss like that with the first round of the Human Mobility Programme. And then recipients had to decide what would happen if they had applied for two posts and had been awarded only half of a post, at a salary comparable with their own whole salary... but nothing for running costs?

I'm sure that it's all much better now, of course. But I'm making a different point. Research is a serious business, and researchers are serious people: they want to change the world and improve it. When they get the impression that there are people in Brussels who have money to spend in a hurry, they feel like monkeys and they act like monkeys. I've got nothing against *real* monkeys, you will understand.

That's all administrative stuff, but it helps to determine the Commission's reputation in the research community. I'd like now to turn to the grand policy issues, and to make a preliminary financial point.

The Commission's fond of saying that it's only a small player in research, and that it spends only a few per cent on what member states spend under the heading of research. That's true enough. But that's not the whole story. The Commission's spending is more important than its monetary value. Rightly, the Commission's research programmes are different in kind from those of national governments, and grants are awarded by different criteria. That adds an element of pluralism to the support of European research that is, in principle, of immense value. I should add that many of the Commission's programmes, Esprit for

example, require that some of the participants should provide matching funds, which further enhances their impact.

I've been talking of the "Commission's programmes" when, constitutionally, that's incorrect; the line items in the various Framework programmes, and in the budgets of the other directorates-general, must be approved by the Council of Ministers, so that they're strictly EU programmes. But since the negotiation of the First Framework Programme 15 years ago, the Council of Ministers is less influential in this field than in, say, agricultural policy. How can it be otherwise, when some member states do not have research ministers and when the assertion of the right of subsidiarity persuades some governments that they will approve the Commission's plans for research only if the Commission plans to carry out projects that they themselves would not support? Where research policy is concerned, the Commission proposes and the Council of Ministers disposes.

The first Framework programme began only 15 years ago, but the Commission acquired a mandate for research only with the Single Act, about six years later. Looking back to the early 1980s, it's easy to see why the Commission acted as it did. Europe's competitors in the United States and Japan seemed to be forging ahead in information technology and telecommunications. So, said the Commission, let there be a scheme to encourage intra-European collaboration in these fields. Interestingly, the first Framework programme was a close echo of what France's Pierre Aigrain had been urging on the European Community at the beginning of the 1970s. The benefits would be that, say, Siemens and Philips would join together in their research in the battle with Sony and AT&T.

The obvious question is why this policy of inducements to collaboration, rational though it may have been in the 1980s, has survived the implementation of the Single Act in the 1990s. Are we not now in the business of waiting to see, in the free market, which companies will drive which others into the ground? Of course, these collaborative programmes do also bring university and small-company groups into adventurous research, but that could be achieved in other ways.

To what else might the Commission devote its energy and funds? I have two general proposals, one practical and one idealistic.

On the practical side, there's the business of service research — public health of animals as well as people, water and air pollution, agricultural research. Europe might save a great deal in talent and in money by the effective coordination of these functions. I should emphasize that by coordination I do not mean centralization, although some of that would probably be found necessary. Simple avoidance of duplication would be a great boon.

And here's the dream. The Maastricht Treaty for the first time gave the Commission competence in higher education and, by implication, for academic research. We all understand, I think, why very little has been done so far. But what if the next millennium's Galileo has the misfortune to be born in Greece, Portugal or Ireland? Isn't a consideration of equal opportunities in science for all the community's young people now part of the Commission's responsibility?

Autonomy

I'm anxious not to be misunderstood. I do not underestimate the degree to which most governments regard their university systems as symbols of cultural distinctiveness. In some places, they've become the equivalents in the 1990s of what national airlines and television broadcasting systems were in the 1960s. Their national autonomy will be powerfully defended by the doctrine of subsidiarity in the years ahead.

Up to a point, that's proper. The last thing that Europe needs is a centrally organized and uniform system of higher education; too many European university systems are too tightly controlled already. Nor do I believe that, politically, the time is ripe for the Commission to develop a policy on higher education for research. But the Commission could perform a great service by strengthening the infrastructure of research in Europe's academic sector.

There's also a grander goal. As things are, there are perhaps half-a-dozen universities in Europe that bear comparison with the other great centres of learning in the world — Harvard, Berkeley, Stanford are the names that come to mind. Increasingly, those centres are international and polyglot; they recruit both academics and students internationally. Europe has the size and talent to function in that plane. The Commission should find a way of creating the conditions for the emergence of truly international universities, the confines of subsidiary notwithstanding.

There remains the problem of the Commission's own relationship with the research community. The Commission's routine work is exceedingly technical — safe levels of nitrate in drinking water, the mechanical strength of bridges and things like that.

In my judgement, the quality of the advice the Commission is given on technical issues is not as high as it deserves. Europe does not lack technical competence, and the research community is not deficient in goodwill towards the Commission. But the Commission is trying to function as if it were a kind of government without the central access to scientific advice that a modern government must have. Putting that right would not cost a lot of money, and would even help to make Europe a place in which science might prosper. Wouldn't that be worthwhile? □

Systematic errors in "Big G"?

The numerical scatter in classical measurements of the gravitational constant may, after all, be explicable; the elastic properties of the wires from which torsion balances are suspended may be frequency-dependent.

THE methods of measurement of Newton's gravitational constant have hardly changed since Henry Cavendish's first successful attempt in 1789. Take a torsion balance, perhaps a metal bar shaped like a dumbbell, suspended through its centre of gravity by a fibre of some kind. Then place equal (and very large) masses symmetrically on either side of the axis of the fibre and with their centres of gravity in the same horizontal plane as the centre of gravity of the bar. Then let the dumbbell oscillate through a small angle, and measure the period or the frequency of the oscillation.

A little thought will show that there are two stable orientations of the torsion bar between the gravitating masses: one in which the bar lies along the line joining the two masses and the other, which is metastable, in which the bar is perpendicular to that line. So the measurement of the frequency of oscillation in each of these two positions is a measure of the gravitational force-couple acting on the bar in terms of the force in the fibre that resists twisting, the torsion force. Two measurements give two equations, from which the supposedly constant force in the fibre can be eliminated. The outcome is a measurement of the gravitational constant G as defined in Newton's equation for the force between two masses, GmM/r^2 (with obvious notation).

There is general admiration of Cavendish (after whom the Cambridge physics laboratory is named) for having won a result of any kind with the techniques available at the end of the Eighteenth Century. In practice, much of the credit for that goes to a country parson, Michell, who moved to London to help the great man, who designed the experimental arrangement and who died before Cavendish's measurement was made.

The passage of two centuries has notoriously failed to bring as much precision to the measurement of G as would be expected for such an important quantity. So much became apparent during the great hunt for the supposed 'fifth force' a decade ago. Typically, individual measurements have estimated errors of one part in a thousand. Disconcertingly, different measurements yield values that differ among themselves by several parts in a thousand, as if there were some systematic error in the measurements or their interpretation.

Now, it seems, a Japanese physicist at the University of Tokyo, Kazuaki Kuroda, has identified a recondite cause of system-

atic error in the estimation of G by means of the time-of-swing of a torsion balance (*Phys. Rev. Lett.* **75**, 2796–2798; 9 October 1995). In essence, his explanation is that the torsion force in the suspending fibre may not be a fixed quantity, but may be dependent on the frequency with which the torsion bar is oscillating. Plainly that would vitiate the assumption that the behaviour of the suspension of the oscillating bar is a constant in the two measurements of the frequency in the parallel and perpendicular configurations respectively. The matter is all the more disconcerting because the variability of the elastic constants is particularly marked at low frequencies (as must be the frequencies of oscillating torsion balances used for the laboratory measurement of G).

By Kuroda's account, the frequency dependence of elastic constants has come to light because of current interest in the design of oscillating masses for use in the detection of gravitational waves when that becomes a reality. Plainly those who hope to practice that trade need to understand the sources of noise in their detectors, which among other things include the dissipative processes that may go on in the material suspending the pendulum bob.

That internal damping in the suspension of a torsion pendulum should influence the dynamics of the material is not surprising. Standard suspensions include such materials as fused quartz and tungsten wires which, with the best will in the world, are made of crystallites of some kind or another and in which dislocations are bound to appear when the materials are stressed, and then at least partially disappear after an appropriate interval. The experimental data about this phenomenon so far gathered appear to relate to esoteric materials such as an alloy of copper and beryllium (see T. J. Quinn *et al.*, *Phys. Lett. A* **197**, 197–208; 1995), but Kuroda reasonably supposes they will be found in all materials used for suspending torsion balances.

To construct a model for the behaviour of a suspending wire, Kuroda simply supposes that the torsional elastic constant will be a complex quantity, the real part of which has its usual meaning while the complex part represents the internal damping. (The torsional constant is simply connected with what is called the shear modulus.) But the concept of damping depends crucially on the existence of a relaxation time, which in turn implies that the behaviour of a suspension wire will vary with the degree of

mismatch between the oscillation period of the torsion pendulum and the relaxation time. To complicate matters further, Kuroda takes over from the experiments with Cu-Be the notion of a spectrum of relaxation times, ranging from less than 10 seconds to more than 5,000 seconds.

In plain language, what this means is that, for any material, there will be a difference between the elastic constants measured at high frequency and those measured in systems oscillating so slowly that distortions have time in which to relax into their original condition. By analogy with materials such as the Cu-Be alloy, Kuroda concludes that the fractional difference between the high-frequency and low-frequency values of the shear modulus will be of the order of 0.02, and will be positive in the sense that the high-frequency value will be greater than that under relaxed conditions. And that in turn implies that the values of G measured by the torsion balance will be greater than the true value of the constant.

There is some supporting evidence for this assertion. One of the classic torsion balance measurements of G is that by P. R. Hey and P. Chrzanowski in 1942. They supported their torsion bar by tungsten wires differently prepared. In one experiment, the tungsten wire was hard-drawn, in the other a wire of the same dimensions was annealed. The two measurements differed by more than one part in a thousand, or by two standard deviations at each measurement. That discrepancy by itself should have suggested, at the time, that the explanation lay in the way the wire had been prepared.

There are two conclusions to be drawn from this argument, one of which is that the torsion balance may not be the best way in which to measure G . It is particularly troubling that the effect of damping in the suspension of the torsion bar seems to have the unilateral effect of biasing the value of G upwards. Kuroda is appropriately modest in refraining from a call to throw all those carefully constructed instruments on the rubbish heap.

The opportunity to which Kuroda points is that of understanding why the measured values of G since the time of Cavendish have failed to converge on a consensus value. With a little luck, it may yet be possible to go back to previous measurements and remove the bias. The result may yet be a dissipationless suspension for use in precision measurements.

John Maddox

New ways of looking at cities

Michael Batty

IN the past ten years, there has been a sea change in the way geographers and planners have begun to think about the growth and form of cities. Top-down approaches based on models that attempt to simulate the entire organization of the city in analogy to classical gravitation are being supplanted by theories emphasizing the way in which uncoordinated local decision-making gives rise to coordinated global patterns which define the size and shape of cities in familiar ways. Cities appear to be yet further examples of self-organizing structures that emerge from local actions¹. Examples have been demonstrated using models based on diffusion-limited aggregation, in which fractal clusters reminiscent of cities with very dominant central business districts can be grown using local rules from a single seed marking the origin of development².

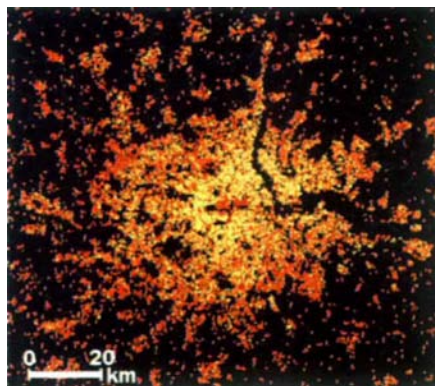
In their paper on page 608 of this issue³, Makse, Havlin and Stanley provide one of the clearest examples yet of such an approach. Their analysis generates forms consistent with power laws describing how population in real cities varies with radius and area, and with relations governing their size and spacing.

Patterns

Cities of course are not single clusters of development centred around their central business districts. They are considerably more complex than this, for even the simplest patterns grow from the amalgamation of clusters formed from small villages, and the world's largest metropolitan regions are composed of many interdependent towns of varying sizes. Models are required that show how such interconnected systems emerge. In moving the model from diffusion-limited aggregation to percolation, and in adding local correlations to achieve free-standing growth of small clusters, Makse and co-workers show how systems of cities with properties that are consistent with empirical observation in large city regions — they use London and Berlin as examples — can evolve. Although this model is somewhat less parsimonious than diffusion-limited aggregation, in that local correlations have to be assumed to generate global correlations, it clearly generates greater realism.

The importance of this general approach to a theory of cities is in its ability to link form to function. Much urban theory developed during the past 50 years has been unable to link the underlying economic and ecological theory of cities to the actual spatial patterns which we observe. The model with diffusion-limited

aggregation shows how widely observed scaling relations such as population density profiles can be linked to simple processes of local growth that give rise to city-like structures. Makse and colleagues' model goes further. It shows how the relations that relate the rank and area of cities to their size, and which are generated by central place theory (the cornerstone of human geography that explains how economic dependence within the hierarchy of cities translates into their location), are entirely consistent with urban form across many scales, from the smallest cluster to the largest urban region. They also show how other types of



Although it is possible to see the morphology of population density in London¹⁰ as a single cluster focused on the central business district, a better explanation of this pattern is as a hierarchy of clusters of different sizes.

scaling, such as the perimeter-scale relation for connected clusters, which is consistent with the notion of a fractal boundary^{2,4}, can be generated. Their work is related to developments in regional trade theory due to Krugman⁵ and to Bak's weak chaos theory⁶, both of which are based on the principle of self-organization which gives this new theory of cities its most informed economic rationale to date. A synthesis is in the offing, and it looks as though the time is now ripe for the new approach to cities and urban form for which we have been waiting for more than a generation.

The implications of the simulations made by Makse *et al.* are wider than the development of new theory *per se*. The principle that underpins their approach is central to the transformation from industrial to post-industrial cities and the ways they might be planned. Cities of the early industrial era were initially single clusters, monocentric forms organized around the central business district. Much of the basic theory that was fashioned in their explanation took this evident simplification, based on the notion that all significant

travel within the city was from homes in the periphery to work in the core, as their starting point⁷.

But as economic constraints on communication have loosened and new technologies emerged, the journey to work is no longer the dominant activity in the city, and cities based on a single core are disappearing. The phenomena of the 'edge city' in North America, and the world city with its global tentacles of communication, are evidence enough that this old vision of the city is passing⁸. Work within the new scheme, based on modelling employing diffusion-limited aggregation, for example², has so far retained the monocentric assumption. But Makse *et al.* break with this, perhaps because they have none of the intellectual baggage which those closer to the field carry — evidence once again that fresh insights in science come from outsiders using established approaches in new fields.

Planning

Most important, in my view, is the impact this change in our understanding of cities may have on planning and intervention. Urban planning, which was institutionalized in western societies over 100 years ago, remains a top-down activity. This view is waning as societies become decentralized, and as central planning based on command economies collapses⁹. Understanding complex systems must be from the ground up as more realistic ways of managing complexity are fashioned. Makse *et al.* suggest, quite rightly, that central planning based on instruments such as the Green Belt policies that have been used to contain London seems to have had little effect on the shape of the metropolis, and that this is consistent with the ways cities are formed from more local actions without any centralized intelligence.

Theories like these are generating changes in our views of cities and how we might alleviate their problems. They are likely to be much more effective than those that have operated hitherto. □

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Making one cell from two

Jeffrey B. Miller

MULTINUCLEATE muscle fibres, or myotubes, are formed by the intercellular fusion of myoblasts¹. Several proteins with extracellular domains, including cadherins, integrins and neural cell-adhesion molecules, are implicated in myoblast fusion², but we do not yet know in detail how the process is carried out. Now, as described on page 652 of this issue³, Yagami-Hiromasa *et al.* have added new candidates — transmembrane proteins termed meltrins — to the list of possible participants in myoblast fusion or differentiation.

The authors searched in muscle cells for genes encoding proteins with sequence homology to the fertilin proteins (fertilins are implicated in the binding and fusion of sperm with egg⁴). They found three such genes, which they call meltrins. Meltrin- α and meltrin- β are newly identified; meltrin- γ was known only from an expressed sequence tag⁵.

Meltrin- α contains a disintegrin and a metalloprotease domain, and as such seems to belong to the protein family known as ADAM⁶. In common with other such proteins, it also has a transmembrane domain, several possible phosphorylation and glycosylation sites, and a region similar to the fusion peptides of fertilin and some viruses. The cytoplasmic domain of meltrin- α , however, is much longer than that of fertilin. Given this domain structure, meltrin- α could reasonably be expected to function in cell-cell fusion and cell-matrix interaction, and in intracellular signalling.

Of the three meltrins, the α form has the most restricted pattern of expression, being found mainly in skeletal muscle and also in bone where multinucleate osteoclasts reside. Because of that, Yagami-Hiromasa *et al.* tested whether meltrin- α could alter myoblast fusion. When transfected into cells of the C2 mouse muscle cell line, the full-length version of meltrin- α slowed fusion, whereas fusion was accelerated by a shortened version that lacked the metalloprotease domain; the shortened protein was tested because muscle cells contain that form as well as the longer version. Because metalloprotease function is required in myoblast fusion⁷, the shortened meltrin- α might be formed by self-proteolysis or in several steps requiring other metalloproteases. Yagami-Hiromasa *et al.* also found that meltrin- α expression begins in

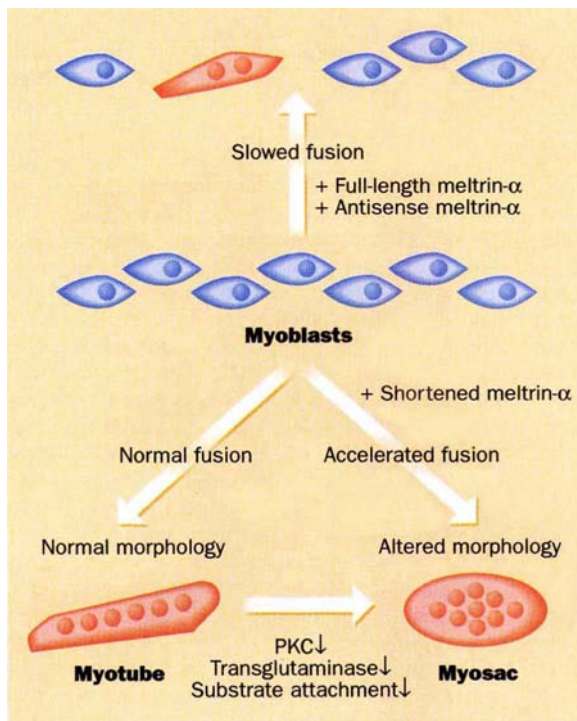
myoblasts at about the same time that fusion starts, and that fusion is inhibited when meltrin- α levels are reduced by an antisense method. Both observations are consistent with the idea that the protein is involved in fusion.

Nonetheless, the function of meltrin- α in myogenesis is not yet established. Myotube formation requires that myoblasts align with and adhere to each other

can by itself induce intercellular fusion⁸. Such simplicity, however, is not universal; for example, it seems that the herpes and human immunodeficiency viruses have more complex requirements for fusion, and that fertilin has to interact with an integrin⁹. Meltrin- α may promote fusion in cooperation with other proteins, perhaps integrins or other ADAM proteins which are expressed in muscle³⁻⁶. Alternatively, it may be involved in myogenic processes other than fusion.

One more clue to the function of meltrin- α comes from Yagami-Hiromasa and colleagues' observation that, as well as accelerating fusion, the short form of meltrin- α causes C2 cells to form multinucleated myotubes with an unusual morphology. C2 myoblasts normally form long, thin myotubes in which most nuclei are arranged in single file. In contrast, C2 cells that overexpress the shortened meltrin- α produce large, almost circular 'myosacs' in which nuclei are randomly distributed or aggregated (see figure). Interestingly, such myosacs also form upon inhibition of protein kinase C, transglutaminase, substrate attachment and microtubule assembly¹⁰⁻¹³. Because normal myotube morphology requires a properly assembled microtubule network, the common effect of these perturbations of cellular processes may be to alter the assembly of tubulin and associated proteins. If so, then one function of meltrin- α , which is consistent with its domain structure, may be to link extracellular events with the intracellular pathways that regulate cytoskeletal morphology.

What next? Analyses of gene knockouts, mutant proteins and the effects of blocking antisera will go a long way towards establishing the functions of the meltrins. Further investigation is also needed to identify meltrin-binding partners and to confirm that the predicted metalloprotease, disintegrin and fusion peptide sequences of meltrin- α are truly functional. Perhaps examination of gene expression in fibroblasts that can fuse with myoblasts¹⁴, or in myoblasts that fuse only under defined conditions^{15,16}, will show whether meltrins or other candidate proteins can be dispensed with



During the normal course of muscle development, myoblasts fuse to form long, multinucleate myotubes. Yagami-Hiromasa *et al.*³ find that, when transfected into C2 mouse muscle cells, full-length meltrin- α slows fusion. Myotube morphology is altered by overexpression of a short form of meltrin- α , as well as by treatments such as inhibition of protein kinase C (PKC), transglutaminase and substrate attachment. The implication of these latter observations is that microtubules and their associated proteins are likely to be the common targets of agents that alter myotube morphology.

before fusion². When non-muscle cells are made to express shortened meltrin- α , however, they do not acquire the ability to fuse, adhere to each other, or undergo myogenesis, meaning that meltrin- α alone probably cannot induce these events. The influenza virus haemagglutinin protein

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during fusion. In the end, we are likely to find that myoblast adhesion, in common with neuronal adhesion¹⁷, requires several systems operating in parallel to guarantee specificity, and that, after adhesion, additional systems must operate in myoblasts to generate the localized physiological changes, including the movement of calcium ions, required for successful fusion^{2,8}.

Although intercellular fusion is carried out by only a few types of cells, including sperm and egg, myoblasts and osteoclast precursors, it is crucial to successful devel-

opment. For myoblasts, we currently have a rather long list of proteins and other molecules that may be involved. More candidates will probably be found, and the challenge is to design experiments that will directly identify the central players in the process and thus open the way to a detailed mechanistic understanding of intercellular fusion. □

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IMMUNOLOGY

Ways around rejection

David L. Vaux

UNLIKE other tissues, grafts of testicular tissues are not efficiently rejected even when transplanted into unmatched hosts¹. Transplantation biologists have longed to understand the reason for this 'immune privilege', hoping that such knowledge would allow methods to be devised to enable patients to avoid rejection of organs from unrelated or animal donors.

On page 630 of this issue², Bellgrau and co-workers show that mismatched grafts of mouse testes, which express the ligand for CD95 (a cell-surface molecule also known as Fas or Apo-1)³, survive, whereas grafts taken from *gld* donors, which lack functional CD95 ligand, are rejected. These results raise the possibility that, in the future, organs engineered to express transgenic CD95 ligand could be used to relieve the chronic shortage of matched donor grafts.

The immune system is designed to recognize and destroy invading organisms. T and B lymphocytes become activated

when their receptors bind foreign antigens. This is usually desirable, but when the foreign antigen happens to be from a transplant it can cause graft rejection (*a* in the figure). To minimize this possibility it is necessary to match the donor and recipient as closely as possible, but such well-matched grafts are hard to come by. Allografts — grafts from donors with different loci at the major histocompatibility complex (MHC) — are more readily available, but are especially prone to rejection because MHC molecules interact directly with the antigen receptors on T cells. Non-human species are potentially an abundant source of tissue (xenotransplants), but these are rejected the most rapidly.

The testes, the anterior chamber of the eye, and the brain have long been known as immune-privileged sites because allogeneic or xenogeneic tissue grafted into them is not rejected; in the brain and the eye this has been attributed to lack of lym-

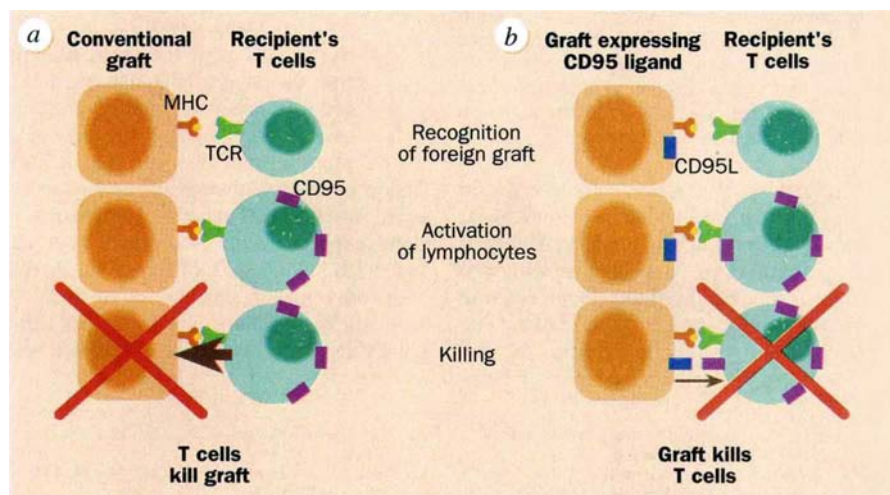
phocytic surveillance⁴. Unlike these sites, testes have excellent lymphatic drainage⁵, and testicular tissue itself is unique as it survives when transplanted into mismatched recipients¹.

Similarly, mismatched grafts of other tissues are rapidly recognized by the host's immune system and are rejected by cytotoxic T cells. Antigens on foreign tissues such as invading pathogens or mismatched grafts are detected by the host's lymphocytes, which then become activated. When T cells become activated, they express CD95 (ref. 6), a cell-surface molecule first identified as the target of a monoclonal antibody that could induce the death of lymphoma cells⁷. It is thought that CD95, which delivers an apoptotic signal to the cell when bound by its ligand, allows the disposal of lymphocytes at the end of an immune response⁸. The *lpr* and *gld* mice, which respectively bear mutated genes for CD95 and CD95 ligand, accumulate large numbers of lymphocytes and tend to contract autoimmune disease, presumably due to failure of lymphocyte death⁹.

Bellgrau *et al.* followed up the observation that CD95 ligand is highly expressed in the testes³. They hypothesized that host lymphocytes can recognize a mismatched testes graft, just as they would recognize any other graft. But when they do, and thus become activated and express CD95, they are promptly killed by ligand on the testes cells (*b* in the figure). To test their hypothesis, Bellgrau *et al.* used *gld* mice, which lack CD95 ligand, as the source of donor testes tissue. Their idea appears to have been correct, because the recipient animals rapidly rejected these grafts, even though they did not reject testes grafts from wild-type donors. Furthermore, preliminary data indicate that *lpr* mice, which lack functional CD95, were able to reject allogeneic testes grafts from wild-type donors.

These findings have implications for transplantation. T and B cells not only reject allografts, but are also the main barrier to xenotransplantation (xenografting is straightforward in SCID mice, so-called because they suffer from severe combined immunodeficiency). Even survival of well-matched grafts in humans often requires doses of immunosuppressant drugs that make the patient susceptible to opportunistic infections. However, by making donor animals that are transgenic for CD95 ligand, it may be possible to use the ligand as an in-built, specific immunosuppressant.

In a patient given such a transplant, those T cells that specifically recognize the graft would become activated, express CD95 and be killed by the graft. The remainder of the recipient's immune system would be unaffected, and remain fully competent to deal with other threats. In this way CD95 ligand would enable the



a, Normally T lymphocytes recognizing foreign tissue are activated when their receptors (TCR, T-cell antigen receptor; green) bind foreign antigens (yellow) associated with the major histocompatibility complex (MHC, brown): this activation results in the rejection of the graft. *b*, Sertoli cells from the testes are protected against rejection because they express CD95 ligand (CD95L, blue), causing the destruction of T lymphocytes expressing CD95 (purple).

graft to respond tit-for-tat: any T cells that attempt to reject the graft would themselves be eliminated.

That's the theory, but there are plenty of questions that must be answered before we will know if such an approach will work in humans. Bellgrau *et al.* show that the expression of CD95 ligand is necessary to ensure the immune privilege of the testis, but will it be sufficient? It could be that other, yet to be identified, factors expressed by this tissue are required for the ligand to exert its action. Moreover, it is not known to what extent organs can become immune-privileged before they succumb to invading pathogens. The answers to such doubts await the generation of mice transgenic for CD95 ligand and the transplantation of their organs into mismatched recipients.

Even if those results are encouraging, grafting organs from pigs to humans will not happen overnight. Thus far the bottleneck in the routine use of animals as a

source of donor organs has not been the aggression of the host's T cells towards the graft: long before the T cells become activated, the graft has been rejected. Natural antibodies circulating in the blood of the recipient recognize endothelial antigens on the graft, and initiate the complement and clotting cascade of the host, resulting in hyperacute rejection within an hour. Although the graft carries its own complement regulatory proteins, the porcine complement inhibitors are not able to keep the human complement in check.

Earlier this year, McCurry *et al.*¹⁰ described the development of pigs transgenic for human complement inhibitors to circumvent this problem; the results look promising, and it seems that the prevention of hyperacute rejection during the critical first 24 hours is well within reach. It remains to be seen whether a genetically engineered porcine graft expressing both complement inhibitors and CD95

ligand will be equipped to fight off the human immune system, or whether it will reveal new obstacles to xenografting. The scientists involved, and maybe ethicists too, are in for a busy time. □

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OBITUARY

Rudolf Peierls (1907–95)

PROFESSOR Sir Rudolf Peierls died on 19 September at the age of 88. He was, I believe, the last of that extraordinarily gifted generation of central European physicists who, having escaped from Hitler's Germany and seeking a career in Britain, took part in the projects for the construction of the atomic bomb.

As his autobiography (*Bird of Passage*, Princeton University Press, 1985) records, it was common knowledge among physicists in the early days of the Second World War that a uranium bomb might be possible. Peierls in 1941 had already achieved the status of professor of theoretical physics in the University of Birmingham, but as an 'ex-enemy alien' he was not involved in military research. He and his compatriot Robert Frisch, also in Birmingham, had the idea of working out from known data what would be the critical mass for an explosion if the pure ²³⁵U isotope could be prepared. Finding — to their surprise — that the answer was only about a pound, they realized that the bomb was possible, and, fearing that Germany might get it first, had no doubt that this information should be communicated to the British government.

This was done and the news was thence passed across the Atlantic, where it was a big factor in convincing the United States to launch a major project. Peierls, in spite of his nationality, was brought into the project, and worked at Los Alamos, as did his assistant Klaus Fuchs, who was later found to have told Russian agents everything he knew. The news was a terrible shock

to Peierls and his Russian wife, Genia.

Theoretical physicists, in the post-war period, have tended to specialize either in nuclear physics and the science of fundamental particles, or in the theory of solids. Peierls, back in Birmingham after the war, refused to do this. He

Edwards and Sir Brian Flowers. He and his wife were indefatigable travellers, going to Seattle most summers, and attending many Pugwash meetings and summer schools.

At home they showed an outstanding hospitality. If someone turned up in Birmingham and could not find a lodging, they could always stay at the Peierls, and if a colleague got married, the reception would always be at the Peierls house.

Peierls is survived by his four children; Lady Peierls died some years ago, and is remembered by the helping hand she gave to everyone. I remember a Pugwash meeting in Cambridge, at which the Russian ladies expected the women in England to be cold and reserved. They had not met Genia yet.

If I say that Peierls is best known for his work on electrons in metals, that is because he wrote a book about this part of his work, and I am a solid-state physicist myself. A paper of his in *Physical Review Letters* last year on vanadium dioxide is entitled "Mott or Peierls?" His achievements include the understanding of a form of metal-insulator transition in one-dimensional systems, diamagnetism in metals and the force resisting the motion of a dislocation. Rudolf Peierls would like to be remembered, justly, as a physicist who turned his hand to any problem.

Nevill Mott

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wanted to be a generalist, publishing papers on applications of quantum mechanics to solids and to nuclei — and to accept research students in both fields. It is extraordinary how many of the senior theorists in Britain and elsewhere have passed through his hands, in Birmingham and later Oxford, including for instance Sir Sam

Hulton Deutsch

Hot times in the solar nebula

Alan P. Boss

How hot did it get? That question was on the minds of meteoriticists gathered in Washington DC this September*. While autumn breezes cooled the Mall, scientists meeting deep underground presented convincing evidence that the early solar nebula was considerably hotter than has been thought likely for quite some time.

The absence of isotope fractionation of potassium in a variety of bodies from the inner Solar System implies that the earliest solids must have formed by condensation in a globally hot nebula with temperatures of 1,200 K or more inside 2 or 3 astronomical units (AU, where 1 AU is the Earth-Sun separation). Theoretical models of the thermal structure of the solar nebula show that such temperatures are plausible, and these models are consistent with astronomical observations of suspected planet-forming disks around many young stars. Experiments, theory and observations thus all seem to be pointing in the same direction, and the model of a room-temperature (300 K) nebula in the asteroid region during the initial phases of planet formation is fast disappearing.

The first serious models of the solar nebula, formulated in the early 1960s, assumed that the presolar cloud collapsed so rapidly that extremely high temperatures (1,800 K) characterized the inner Solar System. The subsequent discovery of centimetre-sized refractory inclusions in the primitive Allende meteorite (which fell in 1969) led meteoriticists to believe that these inclusions were the first condensates that formed as the hot nebula began to cool — their compositions were in remarkable agreement with the predictions of equilibrium condensation calculations. But more detailed calculations showed that the collapse of the presolar cloud could not have occurred as rapidly as was first thought, and that the solar nebula was undoubtedly evolving even as mass was accreting onto its surfaces.

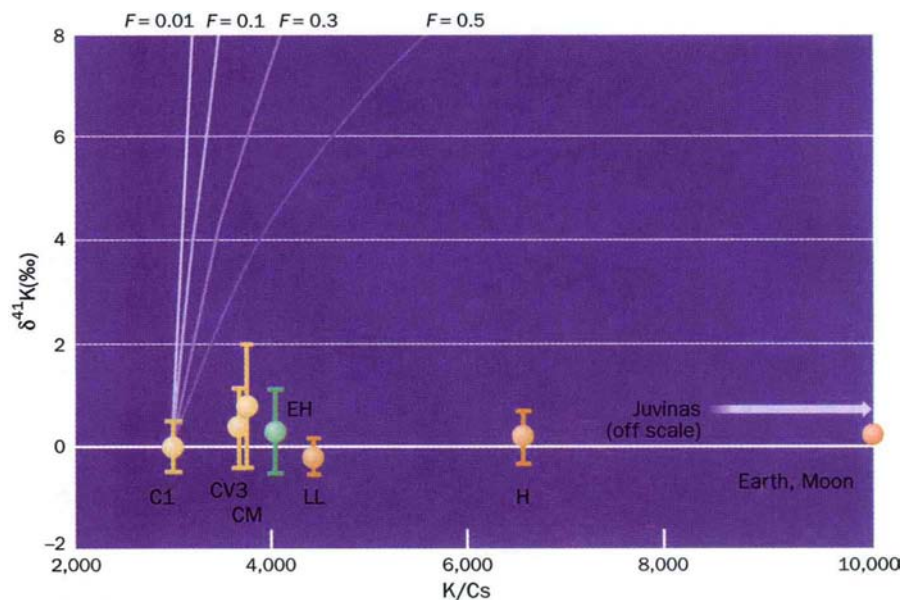
Application of the theory of viscous accretion disks led, at the time, to the conclusion that temperatures as high as 1,800 K at several AU were implausible. Midplane temperatures closer to 300 K at 2–3 AU were predicted for the phase when the mass of the disk had dropped to the so-called 'minimum mass' (that is, the nebula mass that results from restoring the present planets to solar composition by adding the missing hydrogen and helium gas). The formation of refractory

inclusions by condensation thereafter fell from grace, except among the true believers.

Certain refractory inclusions and the millimetre-sized chondrules that give the most primitive meteorites their name (chondrites) have rounded shapes indicative of a molten phase, but non-glassy textures that rule out total melting (see, for example, R. Ash, *Nature News and Views* 372, 219–220; 1994). Laboratory experiments that reproduce chondrule textures have shown that a 'flash heating' mecha-

ately volatile elements such as potassium that are considerably less than that inferred to have characterized the solar nebula. This compositional trend, independent of geochemical behaviour but strongly correlated with volatility, can be explained by thermal processing at about 1,200 K — either by partial condensation in a hot nebula, or by evaporative losses from precursor grains in a cool nebula. Given the accepted picture of a cool nebula, evaporation is implicated.

This happy state of affairs for the cool nebula model was demolished by the determination (M. Humayun and R. N. Clayton, *Geochim. cosmochim. Acta* 59, 2131–2148; 1995) that evaporation could not have been responsible for the de-



The bulk abundances of chondrites can be explained by mixing a devolatilized source with a C1 chondrite source. The curves show the expected heavy isotope enrichment predicted for K if devolatilization occurs by evaporation, as a function of the relative abundance of K (moderately volatile) and Cs (highly volatile) for inner Solar System bodies (the Earth, Moon and Juvinas, a meteorite thought by many to come from the asteroid Vesta) and several chondrite classes (C1, CV3, CM, LL, EH and H). The absence of ^{41}K fractionation rules out evaporation as a devolatilization mechanism. F , the fraction of K remaining in the residue, must be less than 0.5 to account for the K depletion (in absolute terms, not relative to Cs) of these bodies, yet the data points lie far outside this field. (Figure courtesy of Munir Humayun, Carnegie Inst. Washington.)

nism is required. Heating to peak temperatures of about 2,000 K and cooling back below the solidus must have occurred quickly, or else all nucleation sites for the growth of crystalline structures would have been lost and the chondrule would be perfectly devoid of volatile elements as well. Flash heating events are localized in space and time by the restriction to short timescales. The ambient nebula temperature at the time and place when flash heating occurred must have been less than 700 K, in order for iron sulphide to survive in chondrules. This requirement is quite consistent with the cool nebula model — but, as we shall see, it may now be a thorn in the flesh of modellers.

Primitive meteorites (for example, CV3 chondrites) have abundances of moder-

pletion of moderately volatile elements. Evaporation preferentially removes the lighter isotopes of any evaporating species, yet exquisitely precise analyses show that there is no measurable enrichment of the heavier isotope ^{41}K of potassium in any of at least a dozen sampled bodies from the inner Solar System (see also S. R. Taylor, *Nature* 376, 20–21; 1995).

The presence in chondrites of highly volatile elements (such as caesium or sodium) at just fractions of their solar abundances suggests that the bulk compositions of chondrites can be explained by mixing of two components — devolatilized and solar composition (C1) solids. Reproducing the devolatilization trends by mixing C1 solids with residues produced by evaporation in a cool nebula

*Fifty-Eighth Annual Meeting of the Meteoritical Society, Smithsonian Institution, Washington DC, USA, 11–15 September 1995.

leads to predicted enrichments (see figure) that can be ruled out by the lack of evidence for ^{41}K fractionation (M. Humayun, Carnegie Inst. Washington; R. N. Clayton, Univ. Chicago). Incomplete condensation is required, and that requires a nebula at 1,200 K or so.

Other cosmochemical evidence also indicates that condensation was important. An unusual refractory inclusion from the Maralinga CK chondrite with several distinct layers (a core, mantle and crust) seems to have formed from the sequential condensation of silicates from a gas that was already depleted of the most refractory elements (E. Zinner, Washington Univ.). Furthermore, the abundances of rare earth elements in refractory inclusions do not agree with the abundance patterns produced by evaporating bulk samples of chondritic composition (A. M. Davis, Univ. Chicago; A. Hashimoto, Hokkaido Univ.), but have been successfully explained by equilibrium condensation calculations.

Cosmochemistry thus points to a much hotter nebula than that predicted by previous viscous accretion disk models. This disparity has been removed by radiative hydrodynamics models that result in midplane temperatures of 1,200 K at 2 AU in a low-mass nebula still undergoing mass accretion (A. P. B.). The models are consistent with astronomical observations of suspected protoplanetary disks around T Tauri stars, which imply disk surface temperatures around 200 K at 1 AU — because these disks are very optically thick, midplane temperatures must be much higher. When viscous accretion disk models are forced to meet this surface temperature boundary condition, the requisite high midplane temperatures result, and a detailed model of particle coagulation in such a hot nebula leads to predicted abundances for volatile elements in striking agreement with analyses for several chondrite classes (P. Cassen, NASA Ames Research Center).

So was the nebula simply hotter than we thought? No — the need for low ambient temperatures during chondrule flash heating means that chondrites contain components formed from both hot (over 1,200 K) and cool (below 700 K) phases of the nebula. Also, retention of noble gases such as xenon in presolar diamonds requires temperatures of less than 700 K (U. Ott, Max Planck Inst. Chemistry).

One solution to this thermal heterogeneity problem is that refractory inclusions and devolatilized chondrule precursors formed first, when the nebula was still hot, and the other components formed or accreted later, when the nebula had cooled significantly, perhaps 1 Myr later. But there immediately arises the problem of storing particles such as the refractory inclusions while the nebula cools. Gas drag should force the

inclusions to spiral into the Sun instead.

Precisely the same storage problem bedevils our understanding of why the refractory inclusions generally contained live ^{26}Al (see my earlier News and Views article, *Nature* **375**, 13–14; 1995), whereas physically adjacent chondrules did not, implying that chondrules formed so long after the inclusions did (several Myr) that

their ^{26}Al had already decayed. Perhaps a single bright idea will suffice to solve both the thermal heterogeneity and the ^{26}Al heterogeneity puzzles — but what is it? □

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ASTRONOMY

Seeing how galaxies form

Craig J. Hogan

GAS in the early Big Bang was nearly uniformly distributed, as we know from the abundances of light elements and the isotropy of the cosmic microwave background radiation. In broad terms, we also know that this gas formed into galaxies because of gravity: a slightly overdense region of the Universe expands more slowly than the average gas and gradually falls farther and farther behind, until it stops expanding altogether and collapses. Within each galaxy, some of the gas collapses to high density and forms stars, whose production of energy eventually suppresses further star formation and thereby leads to a regulated steady state, in which a galaxy's remaining reservoir of gas is steadily converted into stars over a long period of time.

Until recently, the details of how and when these processes and others moulded the properties of galaxies were obscure, as they had to be pieced together from fossil evidence in the contents and structure of galaxies in the present-day Universe. With new astronomical techniques, however, it is now possible to see so far away that we can effectively look back to the period in the history of the Universe when galaxy formation was occurring. On page 603 of this issue¹, Cowie, Hu and Songaila describe the latest step in this programme, perhaps a watershed: evidence that observations have now reached the critical formation epoch of most of the stars of the main galaxy populations.

Cowie *et al.* apply the light-collecting power of the Keck telescope to collect better spectra of faint galaxies than was previously possible. For many of them — galaxies with strong emission lines — this has led to redshifts being measured, giving a much more precise idea of their distance and of their physical properties, such as the overall brightness, the continuum spectrum in the rest frame and the strength of emission lines. The new survey includes the most distant galaxy ever found by its starlight (as opposed to the light from an active nucleus such as a quasar) which, with a redshift $z = 1.61$, was emitted when the Universe was 2.61 times smaller than it is today.

The most interesting thing about these galaxies is not their record-breaking distance, but the rate at which they are forming fresh stars out of gas. The star formation rate can be estimated from the supply of ultraviolet ionizing radiation in a galaxy, which can in turn be estimated from the amount of light emerging in fluorescent emission lines. Ionizing radiation is a good way to estimate star formation rate because it can be produced only by hot massive stars; these burn out so quickly that if they are found at all in a galaxy we know that they must be constantly replenished by new ones forming out of interstellar gas, at a rate directly proportional to the ultraviolet luminosity. Although there are variations and uncertainties in the conversion factor from line luminosity to star formation rate, the quantitative connection has been thoroughly documented in detail in nearby galaxies².

Cowie *et al.* have found in the new deep spectra a population of galaxies with high star formation rates, high enough that, if the same rate were to continue for the age of the Universe then (about a quarter of what it is now, or a few billion years), each galaxy would have formed all the stars in a typical bright galaxy today. Such high star formation rates are very rare in the Universe today, but appear commonly in the sample taken by Cowie and co-workers.

The observed number of these bright emission line systems is significant: not only are the individual galaxies forming stars fast, but the total production of stars is prodigious. The total volume density of stars formed in just the observed galaxies is 4–20 per cent of all the stars in galaxies today. Adding galaxies whose spectra have not yet been measured (but whose brightnesses and colours are similar) seems likely to double this number; there are also likely to be many individually fainter systems at the same redshift not yet in the spectroscopic sample. (The fraction could also go up if the Universe is open or has a cosmological constant.) It is therefore plausible that we are viewing a substantial portion of the population of

modern giant galaxies caught at the time when they are forming the bulk of their stars out of gas.

This agrees with the picture we get from directly viewing the gas at high redshift rather than the stars. The cosmic inventory of neutral hydrogen gas, measured from damped Lyman-alpha absorption in quasar spectra, includes about the same amount of material as present-day stars at $z = 3$, but a much smaller amount at the present epoch³. This makes sense if the damped absorbers are protogalaxies which form most of their stars between about $z = 1$ and 2. This period should then correspond to most of the cosmic production of heavy elements, also roughly in accord with what is seen in quasar absorbers^{4,5}.

Not only do we now know the distance and the physical conditions in these galaxies, we know what they look like. The Hubble Space Telescope now images them with enough clarity to reveal their detailed morphology. Instead of appearing as generic faint blue blobs (as they do in ground-based images), they show up as having a rich and varied structure strikingly different from most galaxies today. Instead of finding the symmetric disks, well organized spirals, and smooth triaxial shapes most common in bright galaxies close to dynamical equilibrium, we find⁶ irregular patterns, multiple centres of concentration, chains and one-sided arms that characterize galaxies in a period of rapid dynamical evolution, before they have settled into their final quasi-stable regulated steady state.

It now seems likely that many of the galaxies in these images are not faint blue dwarfs at moderate redshifts of the order of 0.5 which were already revealed in deep redshift surveys⁷, but are progenitors of large galaxies like the Milky Way at somewhat earlier times. We are beginning to observe directly how galaxies got the way they are, by viewing in several different ways — spectroscopy, absorption, and direct imaging — the evolution of the structure, chemical enrichment, gas content, star formation history, clustering and other properties of the main population of galaxies in the Universe. □

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Heavy metal revival

Gregory A. Petsko

It is one of the most widely used anti-cancer drugs in the world. It is a cure for testicular cancer if the disease is diagnosed in time. It shows promise for the treatment of ovarian and breast cancers. And there isn't a single atom of carbon in it.

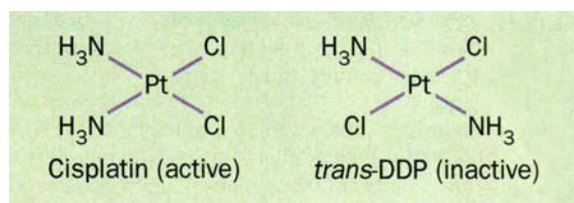
This wonder drug is cisplatin, an inorganic compound first synthesized in 1845. It has been known for years that its cytotoxic effects arise from covalent binding to DNA, but the precise structure of the adduct remained unknown. Now, on page 649 of this issue¹, Takahara *et al.* report the high-resolution crystal structure of a DNA dodecamer containing this adduct. The structure provides insight into why tumour cells may not be able to repair the platinated DNA, and also offers a framework for the design of new anti-cancer drugs.

The serendipitous discovery of the antitumour activity of cisplatin (see figure) is one of the great examples of chance favouring the prepared mind. It is also a classic example of basic research leading to unexpected practical benefits. Barnett Rosenberg was not looking for an anticancer drug. He wasn't even studying platinum compounds. In 1961 he was investigating the effect of an electric field on growing bacterial cells. He used platinum electrodes to generate the field because platinum was supposed to be inert. *Escherichia coli* cells subjected to the electric field stopped dividing and formed long, growing filaments.

Rosenberg knew that chemical compounds producing similar effects on *E. coli* were used as chemotherapeutic agents (yet another moral of this story is that there is no such thing as useless information), so he investigated further. In 1965 he found that the electric field was not responsible for the filamentous growth; rather, platinum from the electrodes, ammonium chloride in the growth medium, and light had combined to synthesize amminechloro compounds of platinum. The *cis* isomer of diamminedichloroplatinum(II) (*cis*-DDP) was found to induce the filamentous growth in *E. coli* and also to be active against implanted tumours in mice. The *trans* isomer (see figure) did neither².

Although Peyrone had first synthesized DDP 150 years ago, and Werner³ described the two stereoisomers in 1893, the differential biological activity of these compounds was both a surprise and a puzzle. First, it was not customary for medi-

cal chemists to think of inorganic complexes as potential drugs, particularly complexes of heavy metals. Second, it was soon established that both isomers formed covalent adducts with DNA. Further studies by Lippard and others (reviewed in ref. 4) showed that cisplatin binds preferentially to purine-rich regions, forming chiefly intrastrand G-G crosslinks, where the two chlorides are replaced by the N7 atoms of adjacent guanines. This adduct cannot be formed by the *trans* isomer, because its chlorine atoms are too far apart (see figure). Instead, *trans*-DDP forms both interstrand and intrastrand



The two stereoisomers of the square-planar compound diamminedichloroplatinum(II). The *cis* isomer (cisplatin) is a potent anticancer drug, whereas the *trans* isomer (*trans*-DDP) is inactive in chemotherapy. The basis for this striking difference may be the ability of HMG-domain proteins to recognize the major adduct of the *cis* isomer with DNA. The structure of this adduct is reported by Takahara *et al.* on page 649 of this issue¹.

crosslinks with one or more intervening nucleotides. Nevertheless, both platinum compounds block DNA replication by virtue of these crosslinks. Why, then, is the *trans* isomer inactive as a drug?

The answer may be that, in the cell, the adducts formed by the *trans* isomer are not processed in the same way as those formed by the *cis* isomer. Although the details are not yet understood, several proteins have been identified that bind specifically to cisplatin-modified DNA. Several of these contain the so-called high-mobility-group (HMG) domain, an 80-amino-acid motif first found in certain non-histone chromosomal proteins⁵. These proteins, which are known to bend DNA duplexes by up to 130°, specifically inhibit the repair of cisplatinated DNA⁶. Observations of this nature have led to the idea that binding of HMG-domain proteins to the cisplatin adduct may shield the complex from excision repair⁷. But what is the structural feature that is being recognized?

Although models have been proposed for the cisplatin-DNA adduct, and X-ray^{8,9} and nuclear magnetic resonance¹⁰ structures have been reported for various platinated oligonucleotides, no high-resolution structure of the double-stranded DNA complex had been determined. Takahara *et al.*¹ have now obtained the

structure of a dodecamer with the intrastrand G-G crosslink in the centre, and the picture is very informative.

The double helix is bent towards the major groove by about 45°. This value is in striking agreement with that determined by Crothers and Lippard for *cis*-platinated DNA by gel electrophoresis¹¹. The central base pairs, including those containing the adjacent G-Cs, are propeller twisted but remain hydrogen bonded. The overall conformation of the duplex changes smoothly but abruptly from the B-form to A-like. This A/B-type hybrid, with its more open and flatter minor groove, may be a recognition element for the HMG-containing proteins. One of the ammine ligands on the platinum forms a hydrogen bond to a phosphate oxygen, which may explain the observed requirement of at least one N-H group for clinically active antitumour compounds. Finally, the platinum atom is significantly displaced from the plane of its guanine ligands, a distortion that might be relieved when an HMG-domain protein binds.

In all, the structure suggests that cis-platin binding to DNA facilitates the conformational changes that must occur when HMG-domain proteins bind¹². Presumably (although this is not yet established), the adducts formed by the *trans* isomer do not have these conformational features.

The structural information presented by Takahara *et al.*¹ suggests that other compounds that induce similar structural changes may have antitumour properties. In the rush to find or create such molecules, it would be well to remember that the rigid, precise geometries of inorganic and organometallic complexes make them attractive candidates for rational design projects. Van Halen and Metallica fans take note: heavy metal may yet be the wave of the future. □

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Insight into intelligence

Nicholas J. Mackintosh

WHAT is intelligence? What does it mean to say that one person is more or less intelligent than another? Are differences in intelligence more or less important than differences in, say, ambition, hard work, ruthlessness, or sheer good fortune in accounting for the success of some and the failure of others? And do IQ tests really measure intelligence — in all or in only some of its aspects? Is it nature or nurture that causes differences in IQ, and how do these causes operate? Do different groups in our society differ in average IQ, and does this matter?

These are seemingly perennial questions, and the fact that they are still being asked suggests a depressing lack of progress. They were very much in evidence at a symposium* organized last month under the auspices of the Galton Institute — ironical, considering that many of them were first asked (and confidently answered) by Francis Galton himself more than 100 years ago.

On one question at least, participants at the symposium were agreed: IQ has significant, even substantial heritability. A cynic might retort that they would say that, wouldn't they? But, in truth, the evidence of the past 20 years or so has made an already pretty well established conclusion well-nigh irrefutable. Two studies of separated identical twins published in the past five years (T. Bouchard, Univ. Minnesota) have reported correlations of over 0.70 in their IQ scores; separated non-identical twins resemble one another much less closely. Several adoption studies have found that biologically unrelated people living in the same adoptive family show less resemblance in IQ than biological relatives. To turn a phrase of Leon Kamin (a well known critic of IQ tests) on its head: no prudent person would now accept that the heritability of IQ was zero.

That question is settled and there are now more interesting questions to ask. Thus we may not know precisely how any genetic effects are mediated, but two conclusions are emerging. First, some genetic effects are mediated in a very roundabout way via the environment: a particular genetic make-up may predispose someone towards selecting, or fashioning for themselves, a particular kind of environment that then works back to affect their IQ. Second (as noted by P. McGuffin, Univ. Wales), molecular genetics research has demonstrated that a continuously variable trait (as IQ is) may be influenced by surprisingly few genes, with two or three

doing most of the work and two or three more completing the picture. No one, of course, is even close to identifying a single major gene with a powerful influence on IQ within the normal range, but this idea may not be in the realm of science fiction.

But behaviour genetics has more to say than this. For example, if the heritability of IQ is not much more than 50 per cent, then something like half the variance in IQ can be attributed to environmental factors. But what are these environmental sources of variation? The standard answer has always been family background. Indeed, critics of IQ tests have usually denounced them as thinly disguised measures of conformity to white, middle-class values, and argued that differences in IQ are simply a consequence of the large difference in social, cultural and educational opportunities permitted by an unjust society. But (as argued by T. Bouchard and by R. Plomin, Inst. of Psychiatry, Univ. London) there is increasing evidence that, as children grow older, so the environmental influence of family background on IQ becomes less important. For example, adopted children brought up in the same adoptive family may show a modest resemblance in IQ when they are young, but none at all by the time they are teenagers. The implication is that, by this age, the major source of environmentally induced variance in IQ occurs within families, rather than between one family and another.

As with many discoveries in social science, it is easy to remark in retrospect that this is tremendously unsurprising. No doubt. All parents know that they do not treat all their children in the same way. But what is being implied is that it is these differences in individual interaction, probably coupled with experiences that growing children create for themselves outside the family, that eventually exert more influence on IQ than do global measures of family background such as those associated with social class.

Behaviour genetics have, paradoxically, probably contributed to our understanding of environmental influences on IQ as much as direct studies of the environment itself, which have yielded an ever-lengthening list of factors with small and sometimes only short-lived effects. Only rarely does a study advance our understanding of how the factor in question exerts its influence. Thus there is evidence (R. Morley, Univ. Cambridge) that infants fed on breast milk rather than formula feeds develop higher IQ scores, but it is not clear what the magic ingredient is, let alone what its more immediate effects are.

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Infectious diseases, parasitic worms or poor nutrition in developing countries (R. Nokes, Univ. Oxford) do not always significantly affect test scores or educational attainment. The only environmental factor that seems to have a reasonably large and long-lasting effect on children's IQ scores is adoption into a nice, middle-class family.

Of course, adoptive homes represent a rather restricted set of environments: criminals or drunkards are not normally eligible to adopt children, and some people who choose to adopt may be more committed parents than others who, so to speak, have children thrust upon them. Thus it is significant that even within this restricted range, the social class of the adoptive home shows a modest correlation with the adopted child's IQ — although there are no longitudinal data establishing that adoption exerts a long-term effect on children's test scores.

One contentious claim is that various groups differ in average IQ — recently extended to include the two sexes. Francis Galton, like many men of his generation, had no doubt that women were the intellectual inferiors of men. But IQ tests have usually been considered to lend little support to male chauvinism, and the undoubted fact that women tend to have smaller brains than men was attributed to a difference in average body size. More recent examination of this evidence has suggested that this is not true: even when the comparison is between men and women of the same size, women tend to have smaller brains. This, coupled with recent evidence of a significant correlation between brain size and IQ, has revived the suggestion that there must be a sex difference in intelligence¹. Whether there really is such a difference may never be answered, for the fact is that while men outscore women on some IQ tests, women outscore men on others, and on some there are no reliable differences worth speaking of. It is obvious enough that different tests must be measuring rather different things. But which is the true measure of intelligence?

One answer, of course, is that there is no single true measure of intelligence because there is no single thing called intelligence — only a number of separate, independent abilities^{2,3}. Most IQ testers acknowledge that there are distinctions between tests of verbal and spatial ability, abstract reasoning and speed of information processing, but point out, quite correctly, that these tests all correlate positively with one another. It is, therefore, at best misleading to say that these tests measure wholly independent abilities. More plausibly, they measure a set of overlapping processes whose importance varies from one kind of test to another. Whether there is a single, underlying

process of general intelligence that is more important than the others is simply not known, and dogmatic pronouncements from critics and supporters alike are misplaced.

There is no dispute that some groups differ substantially in average scores on virtually all kinds of IQ test — the most widely publicized example being that between blacks and whites in the United States. Those who study such differences, and have argued that they are probably in part genetically mediated, have sought to defend themselves against a charge of racism by insisting that, in a just society where people are judged as individuals rather than as members of a group, differences in average IQ between groups would be of no consequence. A forceful counter-argument (J. Flynn, Univ. Otago) is that people do, and are virtually bound to, judge others as members of groups: car insurance companies and police officers take a different view of young males and of middle-aged, respectable females, and if the young male is black, their view will be even more jaundiced. Of course this leads to much injustice but it is also understandable and perhaps even rational: the fact of the matter is that young male drivers are relatively bad insurance risks, and young male blacks commit more crimes than some other sections of society. Viewed from one perspective, therefore, affirmative action is no more than society's recompense for other, well-nigh inevitable injustices.

One of the theses of *The Bell Curve*⁴ was that affirmative action should be abandoned. But the central argument of that widely denounced book was simply that IQ scores matter: whatever IQ tests measure, whether or not they capture everything that everyone might mean by intelligence, IQ does predict educational and occupational success (and much else besides), rather better than the popular alternatives of social class or family background. There is probably a fair measure of truth in this observation. *The Bell Curve* may be criticized for oversimplification, in particular for frequently falling into the trap of assuming that prediction or correlation implies causation. But the sociology of IQ testing will not achieve the maturity now evident in the behaviour genetics of IQ until social scientists start seeking to understand this correlation rather than continuing to wish it away. □

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DAEDALUS

Travelling wave

In the popular sport of surf-riding, the rider stands on an exposed board which slides down the face of an advancing wave as fast as the wave itself travels. Much skill is needed to maintain the board's position on the wave, as it is always in unstable equilibrium. Daedalus once proposed a inverse form of the sport, 'inverted surfing'. This required a buoyant but submerged board, positioned under the water surface at the back face of the moving wave. It kept pace with the wave from behind, by constantly tending to float up to its crest. It too would be unstable; much skill would be needed to keep it in position. For consistency, felt Daedalus, it should be ridden from beneath by a rider suspended upside down from it and wearing an aqualung.

Not surprisingly, inverted surfing never rivalled the conventional variety. But Daedalus now plans to combine the two. A normal surfboard on the front face of a wave, coupled to a inverted board on the back face, should be quite stable. The combination would straddle the wave like a pitched roof, and travel with it dependably, with no need for human skill.

Surfers may sneer at such an unsporting craft; but the invention is not aimed at them. Daedalus sees it as an unmanned ocean research vehicle. Without the drag and loading imposed by a human cargo, it could ride not merely the steepened breakers of a shelving beach, but the broader, shallower ocean waves themselves. Daedalus's 'straddle-surfer' oceanographic platform will be carried out to sea on a boat, and launched onto a selected wave. It will ride away on it at the typical wave speed of 20–80 km hr⁻¹, sharing its path and fate. It could relay its position, together with the local temperature, salinity, wind and so on, by satellite radio. When the wave reached shore, maybe thousands of kilometres away, the straddle-surfer would be cast up on the beach. A public-spirited finder might even deliver it to the local coastguards for return to the sender.

Straddle-surfers would be so cheap that dozens could be launched together on successive waves as a sort of convoy. The distance between them (measured perhaps by on-board radar or satellite geodesy) would usefully give the wavelength of the swell. A few might lose lock on their waves and be left wallowing, but the rest would travel on. A regular traffic of straddle-surfers might even become a new oceanic resource. Sea-birds could take to riding them, as a more restful form of migration. And those wistful souls who send messages in bottles might turn to small, fast-running straddle-surfers as a form of express post.

David Jones

Baseball teams beaten by jet lag

SIR — Rapid travel across multiple time zones can lead to a constellation of symptoms known as 'jet lag'^{1,2}. The severity of jet lag and the number of days required for full recovery depend on the number of time zones crossed and the direction of flight, with faster readjustment after westward than eastward flights. Although jet lag has been investigated in laboratory and field studies of individuals, its economic and social consequences in natural society have been difficult to analyse. Competitive athletics may provide a unique experiment for this purpose. An eastward flight across six time zones diminishes strength and endurance³, and there are a few anecdotal reports that ascribe poor performance in athletic competitions to jet lag^{4,5}. It has not, however, been possible to separate the deleterious effects of jet lag from those due to the stress and fatigue of the flight itself or to the necessity of competing in an unfamiliar environment. Games lost by a travelling team tend to be attributed to the

opponent's 'home field' advantage.

To control for these variables and to gauge the possible effects of jet lag in professional athletics, we have studied the last three complete season records (1991–93) of the 19 North American major league baseball teams based in cities of the Eastern and Pacific time zones. We chose baseball because of the necessity for repeated road trips of about two weeks in length requiring air travel over three time zones, and the fact that only one or two days (at most) separate pre- and post-travel games. In baseball, the large number of games per season, somewhat repetitive activities in each game, and meticulous record-keeping all tend to make long-term effects more easily discernible. As these 19 teams won more of their 1991–93 games when playing at home than away (54% against 46%; $P < 0.0001$; χ^2), we investigated whether this home-field advantage was further influenced by transcontinental travel.

Schedules and box scores were obtained

for the 1991–93 seasons from the American and National League Box Scores and Official Averages. For the target teams, statistical data were collected for the two games immediately before and after each transcontinental trip. The first two post-travel games were used because of the conventional rule of thumb that resynchronization of jet lag requires about one day for each time zone passed. Transcontinental trips interrupted by play in cities of the Central time zone were not included. For the three-year span, the overall winning percentages of the eastern and western teams were no different (50% and 49%, respectively). The home team won 56% of the games we studied, but the probability of winning depended on whether the visiting team had just travelled eastward (Table 1). Further analysis of the influence of various factors on game outcome (Table 2) showed that the home team could expect to score 1.24 more runs than usual when the visitor had just completed eastward travel ($P = 0.006$). Home teams also scored 0.62 more runs during the day than the night ($P = 0.03$). No other factors were significant.

Many factors undoubtedly contribute to winning baseball games, but our data indicate that one critical, previously unrecognized component of the 'home field' advantage of east and west coast baseball teams involves previous transcontinental travel by the visiting team within the preceding two days, but only if the direction of travel is eastward. This effect cannot be attributed to the stress and fatigue of either high-altitude flight or prolonged stays away from home, because home-field advantage is not enhanced after westward travel or at the end of road trips. The dramatic directional dependence is consistent with previous studies of jet lag, in which greater east-bound effects seem to be independent of relative flight orientation (homeward/outgoing) or time of flight departure (day/night)⁶. Whether due to jet lag or some other aetiology, our findings are of practical importance for the west coast teams, because only they face the double handicap of playing their away games after eastward trips. The result is that these teams are giving up more than one additional run in every game played after such travel.

While the performance decrements described here might seem small in magnitude, their consequences for competitive athletics are substantial. For west coast baseball teams, the games we studied represent less than 5% of their entire season; yet, in 1991 and 1993 the National League Western division races were lost by west coast teams to their eastern rivals by only one game.

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. Priority will be given to letters of fewer than 500 words and five references.

TABLE 1 Home team winning percentage depends on the direction of visitor's transcontinental travel

Visitor's direction of travel	No. of games	Games won	Winning %
No travel	712	385	54.1
East→west	194	109	56.2
West→east	175	110	62.9
Totals	1,081	604	55.9

TABLE 2 Linear regression analysis of runs scored

Variable	Parameter estimate	Standard error	P value
Intercept	1.31	0.80	0.10
National or American League	0.03	0.25	0.90
Month	*	*	0.35
Night game	-0.62	0.28	0.03
Coast:			
Home team	-0.13	0.34	0.71
Visitor	-0.15	0.23	0.51
Home team's travel:			
East→west	0.07	0.40	0.87
West→east	0.17	0.40	0.68
Visiting team's travel:			
East→west	0.66	0.40	0.10
West→east	1.24	0.45	0.006
End of visitor's road trip	0.03	0.95	0.47

Game outcome was defined as the difference between the runs scored by the home team and the runs scored by the visiting team. This was modelled by linear regression using the predictors shown. The parameter estimates in the linear regression have a direct interpretation as the number of runs scored. A logistic regression analysis using the binary outcome 'win or lose' confirmed the results assigning significance at $P < 0.05$. Exploratory analyses of interactions among these factors showed no additional significant predictors of runs scored.

*Month of play (April to September) has 6 categories and becomes 5 binary variables in the regression model. Hence, this factor does not have a single regression coefficient.

Uncertainty over complementarity?

SIR — Recent publications in *Nature* and elsewhere¹⁻⁶ have shown complete disagreement between two respected groups of quantum optics researchers over the answer to the question, "Is complementarity more fundamental than the uncertainty principle?" There are a number of points of conflict between the two groups; our aim is to elucidate the reason for the divergence in their opinions on the particular issue outlined below.

The dispute originally centred on a gedanken experiment proposed by the group of Scully *et al.*¹. This variation on the Young's double-slit interferometer has two microwave cavities acting as *welcher Weg* ('which path') detectors capable of determining which slit a particle went through. Scully *et al.* claim that there is no random momentum transfer by the microwave fields, so the loss of interference cannot be accounted for by the uncertainty principle. They conclude that complementarity is the more fundamental concept.

In contrast, Storey *et al.* point out that the loss of interference can also be seen as due to a random phase change induced by the detectors². They claim, on the basis of a general theorem, that the physical interpretation of this phase randomization is momentum transfer in excess of $\hbar/d$, where d is the slit separation. Hence, they give priority to the uncertainty relations.

Our reconciliation of the arguments relies on the recognition that there are two different views on what constitutes a random momentum kick. Storey *et al.* define it as a convolution of the momentum wavefunction of a particle $\tilde{\psi}(p)$ with a momentum amplitude transfer function $\tilde{O}_\xi(p)$, where the parameter ξ is the measurement result, according to:

$$\tilde{\psi}_f(p) = N \int dp' \tilde{\psi}_i(p-p') \tilde{O}_\xi(p') \quad (1)$$

where $\tilde{\psi}_i(p)$ and $\tilde{\psi}_f(p)$ are, respectively, the initial and final momentum wavefunctions, and N is a normalization factor. Classically, however, a random momen-

tum kick would result in the convolution of the momentum probability:

$$P_f(p) = \int dp' P_i(p-p') \Omega_\xi(p') \quad (2)$$

It is not difficult to verify that the two concepts of random momentum transfer (equations (1) and (2)) are not equivalent unless $\tilde{O}_\xi(p)$ is nonzero only at a single point; that is, only if there is a unique value of momentum transfer for each ξ . For well-known *welcher Weg* schemes such as the Einstein recoiling slit and Feynman light microscope⁷, it is possible to find a measurement basis for the detector such that $\tilde{O}_\xi(p)$ has this property, so that the loss of interference may be interpreted in terms of uncontrolled classical momentum kicks. This momentum disturbance could be seen in the broadening of the diffraction pattern from a single slit.

For the scheme proposed by Scully *et al.*, however, there is no way of measuring the apparatus (the state of the microwave cavities) such that an exact momentum transfer occurs for each result ξ . Thus, although there must be some momentum transfer greater than $\hbar/d$ according to the definition of equation (1) (as shown by Storey *et al.*),

there is no random momentum kick in the sense of equation (2), as in previous double-slit experiments. This feature is what makes possible the true novelty in the proposal of Scully *et al.*, namely, that there would be negligible disturbance of the momentum of a particle passing through one slit only. Our analysis should allow similar schemes to be easily identified.

In his debates with Einstein, Bohr used the simple picture of uncontrolled classical momentum kicks to show how the uncertainty principle enforced complementarity⁸. Scully *et al.* have shown that this naive realist interpretation of the uncertainty principle does not work in general. There is thus room for Scully *et al.* to claim that complementarity is more fundamental than the uncertainty principle; but there is also room for claims by Storey *et al.* that one can always consider complementarity as being enforced by the uncertainty principle, if instead the latter is interpreted in terms of the more subtle idea of momentum-kick amplitudes.

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Masculinization costs in hyaenas

SIR — Female baboons (*Papio cynocephalus*) of high social rank may suffer increased rates of miscarriage and infertility compared with lower-ranking females^{1,2}. Among spotted hyaenas (*Crocuta crocuta*), adaptations related to dominance seem to entail even more severe reproductive costs, affecting all social ranks. Females of this species display a unique syndrome of behavioural aggressiveness and genital masculinization that is associated with massive prenatal androgen exposure^{3,4} and is thought to be an evolutionary response to intense feeding competition^{5,6}. Although aggressiveness confers a major fitness advantage to high-ranking females⁷, the associated genital masculinization may exact a significant fitness cost.

Spotted hyaenas give birth through the hypertrophied clitoris, similar in size and structure to the male penis⁸. As an adaptation to the severe fighting that occurs between littermates at birth⁹, neonates are large and developmentally precocial compared with other carnivores. Maternal and neonatal mortality in primiparous

females (those giving birth for the first time) sometimes results when these large fetuses are squeezed through the narrow peniform clitoris.

A typical mammal birth canal extends from the cervix through the pelvis to the external vagina in a relatively straight line. The spotted hyaena birth canal, however, makes a 180° degree bend within the pelvis, and then extends anteroventrally to enter the clitoris (see figure). The birth canal, at 60 cm, is twice the length of that

Calculation of the reduction in potential reproductive success caused by maternal and neonatal mortality in primiparous spotted hyaenas

From field data:

Mean reproductive lifespan of females that survive first reproduction, $L = 8.2$ yr ($n = 32$)
Minimum age at which a cub may survive death of the mother, $s = 1$ yr
Mean interlitter interval, $i = 1.34 \pm 0.03$ yr ($n = 56$ intervals)
Probability of primiparous maternal mortality, $p_m = 0.088$

From captive data:

Mean size of first litter, $n_1 = 1.64$ cubs ($n = 11$ litters)
Mean size of subsequent litters, $n_2 = 2$ cubs⁹
Probability of primiparous neonatal mortality, $p_n = 0.61$
Probability of maternal mortality in primiparae, $p_m = 0.182$

Thus:

Potential reproductive success in absence of mortality due to dystocia in primiparae,
 $RS_p = n_1 + n_2((L-s)/i) = 12.4$ offspring
Realized reproductive success after mortality due to dystocia,
 $RS_r = (1-p_m)(RS_p - n_1 p_n) = 10.4$ offspring
(based on field mortality estimate)
or 9.33 offspring
(based on captive mortality data).

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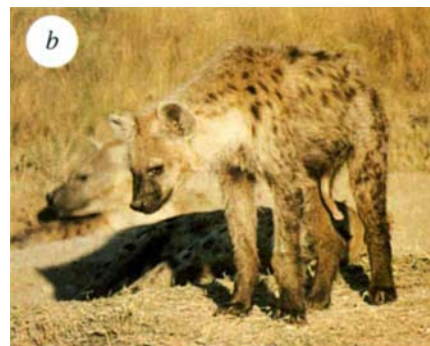
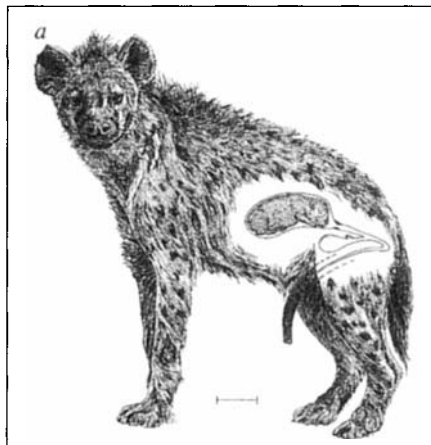
in a typical mammal of similar size. The umbilical cord is only 12–18 cm long, so the fetus dies of anoxia in the birth canal if it is not born immediately after placental detachment.

Labour in primiparous female spotted hyaenas takes many hours, in part because the clitoris must stretch to accommodate the fetus¹⁰. At a diameter of only 2.2 ± 0.8 (s.e.) cm, the clitoral meatus in post-pubertal females is too narrow to pass the fetal head, which is 6–7 cm in diameter. The meatus eventually tears to allow passage, but the fetus may remain lodged in the clitoris for more than 30 minutes before emerging. The meatus remains enlarged and subsequent births to multiparae are rapid and uneventful.

Wild females give birth in seclusion, and birth has rarely been witnessed; therefore, indirect measures are needed to estimate both maternal and neonatal mortality. In our laboratory colony, parturient females are allowed access to a small birth 'den', isolated from other hyaenas and observers to minimize stress. Four out of 11 (36.4%) primiparous females experienced dystocia (abnormal labour) that required veterinary intervention, saving the life of the mother in at least two of these cases (18.2%)¹⁰. Among 12 births to parous females, there were no cases of dystocia (pregnancies occurring after a female had a caesarean section were omitted from the data).

Mortality of wild primiparous females can be estimated from data on 102 wild females born between 1979 and 1990 in a long-term study population⁷. Mortality was 15.4% in the year before age of first reproduction (YFR: 2.5–3.5 years of age), rising to 20.8% during the YFR and falling to a mean rate of $8.6 \pm 3.5\%$ thereafter. Thus, mortality during the YFR increases above juvenile levels, falling to adult levels only in the following year.

Predation mortality during the YFR may be conservatively estimated as the mean of the mortality rates in the year before and years after YFR, yielding an estimate of 12%. Thus, excess mortality attributable to first birth in wild hyaenas is about 8.8%. Both dystocia and predation by lions (*Panthera leo*)⁷ during protracted labour may contribute. Because males disperse at 2–4 years, male mortality rates are not available as an independent estimate of age-specific predation rate. For comparison, female lions in this ecosystem start breeding at the same age as



a, Pregnant female spotted hyaena, showing the path of urogenital canal through the pelvis and clitoris. Distance from uterus to end of clitoris, 60 cm; length of umbilical cord, 12–18 cm. Scale bar, 10 cm. Drawn by C. Drea.
b, Juvenile female displaying erect clitoris.

hyaenas, but show low adult mortality rates before reaching the YFR¹¹.

Data on litter size in primiparous females and on neonatal mortality are available from the Berkeley colony. Eleven of 18 neonates (61%) were still-born due to anoxia during protracted deliveries. Among the parous females, however, there were only two stillbirths (the runts in triplet litters) among 30 neonates. Because dystocia was seen only in primiparous females, it does not seem to be an artefact of captivity, although lack of sustained vigorous exercise in captives may contribute. Females of all social ranks were affected. Fetuses of captives are no larger than wild ones: mean weight at birth of 23 fetuses in Berkeley was 1.33 ± 0.027 kg, while two term fetuses collected in northern Kenya each weighed 1.30 kg. Among captive females of the non-masculinized striped and brown hyaenas (*Hyaena hyaena* and *H.*

brunnea), postnatal mortality due to maternal neglect or abuse is common, but natal mortality due to dystocia has not been documented ($n=52$ and 35 litters, respectively)^{12,13}.

Reduction in potential reproductive success may be estimated using field data for demographic parameters and captive data on births (see box). Based on conservative mortality estimates from field data, dystocia costs the average female two of her potential 12.4 offspring (16.1%). If the 18.2% 'mortality' among primiparous captives is more representative, estimated fitness reduction due to dystocia rises to 24.8%. These estimates require corroboration from observations of births in the wild.

Adult female spotted hyaenas gain a large fitness payoff from the aggressiveness associated with prenatal androgenization. Reproductive success of the highest-ranking females is 2.5 times greater than that of all lower-ranking ones⁷. Dominant females benefit from priority of access to highly contested food³, improved survival of offspring and by conferring high social rank on their daughters. Selection for female aggressiveness apparently counters the mortality costs attributable to the associated genital masculinization.

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Neanderthal infant burial

SIR—One of the most controversial subjects in the study of the evolution and dispersal of modern *Homo sapiens* is the interpretation of the chronological and phylogenetic relationship between skeletal remains classified as Neanderthals and those classified as early modern humans, as found in sites such as Kebara and Amud (Neanderthal) and Qafzeh and Skhul (early modern). The discovery of a Neanderthal infant deliberately buried at the Dederiyeh cave in Syria¹ (Fig. 1a) sheds further light on this.

One theory is that modern *Homo sapiens*, associated with a Tabun C-type Mousterian industry, appeared about 100,000 years ago in the Levant and coexisted with Neanderthals who arrived at a later date, associated with a Tabun B-type Mousterian². A second theory has local Neanderthals and early modern people as variants of *Homo sapiens* in the Levant^{3,4}. Alternatively, the Middle East has been proposed as an area of overlap between European Neanderthals and African-derived modern humans⁵.

The Dederiyeh cave is located 400 km

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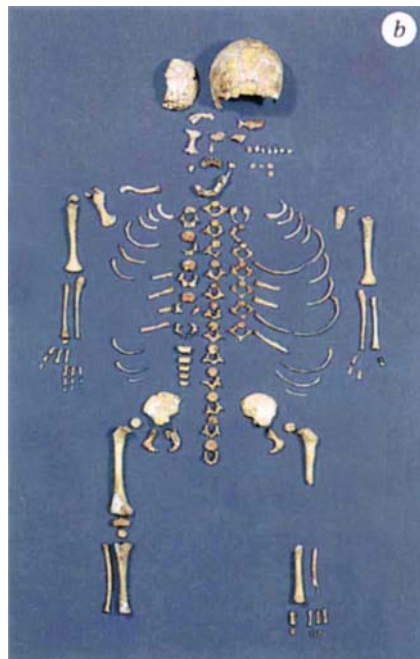
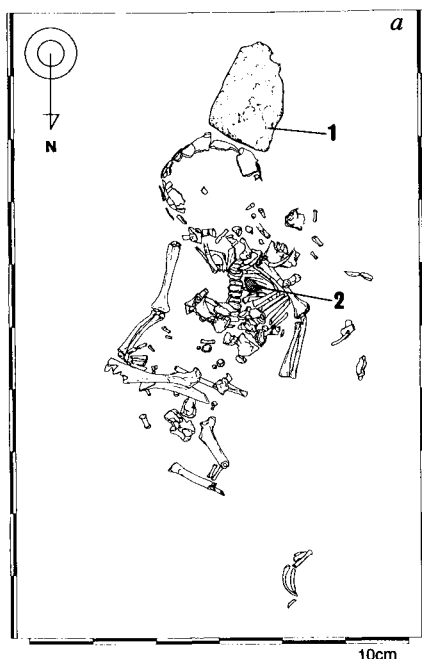


FIG. 1 *a*, Plan of the burial of the Dederiyeh infant. 1, Limestone slab; 2, artefactual flint. *b*, Preservation of the Dederiyeh infant skeleton. Although the major portion of the frontal bone, parts of the facial skeleton, the left clavicle, most of the left hand bones, the ischium and the greater part of the foot bones are missing, many other bones of the skeleton are well preserved. All the vertebrae and the ribs of the left side are perfectly identified.

north of Damascus and 60 km northwest of Aleppo. The cave is providing the best evidence yet of Neanderthal burial practices, as well as data on the morphology of Neanderthals and the chronological position of human types in Levantine Mousterian contexts. The infant was found *in situ* in the Mousterian deposit, lying on its back with arms extended and legs flexed, indicating an intentional burial. A sub-rectangular limestone slab at the top of the head and a small piece of triangular flint just on the infant's head were found in the most sterile layer of the burial fill. A limestone slab of this type is rare in the Dederiyeh cave deposits.

The skeleton of the Dederiyeh child is remarkably well preserved (Fig. 1*b*). From the many morphological features shared with European Neanderthal infants, the Dederiyeh Mousterian child is certainly a Neanderthal.



FIG. 2 The mandible of the Dederiyeh infant. The symphyseal surface recedes inferiorly, with only a slightly developed mental trigone. The mental foramen is duplicated. The submental notch and anterior marginal tubercle are seen at the lower border. The lateral milk incisor, milk canine and second milk molar have not fully erupted.

The following are the chief diagnostic morphological features.

Neurocranium: Globular outline when viewed from behind; presence of a suprainiac fossa and transverse torus in the occipital bone.

Face: Wide and strongly protruding nasal bone, and swept-back appearance of the zygomatic bone.

Mandible: Receding symphyseal profile, with only a slight indication of the mental trigone; two mental foramina on each side.

Teeth: Shovelling of the upper central permanent incisor; strong tendency to taurodontism in the lower first milk molar.

Postcranial bone: Relatively long clavicle; acetabulofemoral elongation of the pubis; strong anteroposterior bowing of the shafts of the femur and tibia⁶⁻⁸.

Stages in dental eruption and calcification in the Dederiyeh infant (Fig. 2) are even earlier than those of Pech de l'Azé^{7,9}, the youngest Neanderthal specimen found so far possessing a relatively complete cranium. According to the classic Schour/Massler chart and Smith's recent ageing method based on dental development^{10,11}, the Dederiyeh infant is considered no more than two years old at death, but the maximum breadth of its cranium (132 mm) is equivalent to that of a modern Japanese six-year-old¹². This may indicate that the Dederiyeh infant had a relatively large brain size for its age, as

did the Devil's Tower and Engis Neanderthal children^{13,14}.

The infant was found in layer 8 of the Mousterian deposit, about 1.5 m from the surface. An analysis of the lithic assemblages of layer 8 indicates a great similarity between the Tabun B-type association found at Dederiyeh and those of the Levantine Mousterian, or the Kebara Neanderthal. Apart from the new discoveries at Dederiyeh, the Qafzeh and Kebara caves are the only other sites in the Levant for which there are clear stratigraphic associations between hominid fossils and well-defined lithic industries^{15,16}.

The Dederiyeh cave has thus produced new data on the stratigraphic association between Neanderthals and Tabun B-type Mousterian in the Levant, and on the distribution of the stratigraphic association between Neanderthals and Tabun B industry in the northern extremity of the Dead Sea Rift. It implies the difference in geographical distribution between Neanderthals and early modern humans suggested by the distributional difference between the industries of Tabun B and C types¹⁷.

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Anatomy of melancholy

Howard Gardner

An Unquiet Mind: A Memoir of Moods and Madness. By Kay Redfield Jamison: *Knopf*. 1995. Pp. 240. \$22.

This first-person account of manic-depressive illness is a riveting read. I devoured it at a single sitting and found the book almost as compelling on a second reading a month later. To anyone unfamiliar with this frightening disorder, which leads to the death of thousands of people annually, I can recommend this book without hesitation. And it also harbours dividends for those in search of fresh insights into what has come to be called bipolar disorder.

Kay Redfield Jamison led a comfortable, almost storybook childhood in the Washington DC area in the 1950s. Her first inkling that she might not be completely in her right mind came after she moved to California as a teenager. At about the time that her father began to behave strangely and her parents' marriage slowly unravelled, Jamison felt unhappy and alienated from her peers and her surroundings. She "soared" and "crashed" repeatedly in college — oscillating between incredible energetic heights and profound despair with her life — but made it through nonetheless. Discovering that her passion was psychology, she proceeded by a fairly direct route to graduate school, a first marriage, a doctorate in clinical psychology and a job as a junior professor at the University of California at Los Angeles. As she declares: "Within three months of becoming a professor, I was ravingly psychotic".

The heart of Jamison's book is a description of her bouts of madness over the next 15 years. It is a chilling story. Despite her interest as a professional in manic-depressive disorders and despite exhibiting textbook symptoms, the youthful scientist goes to great lengths to deny that she is desperately ill. She launches wild physical and mental adventures, embarks on crazy spending sprees, experiences hallucinations of catastrophes, has horrible bouts of depression, hurts those whom she loves and who love her, flirts with various forms of suicide and almost succeeds in killing herself through a massive drug overdose. Prescribed lithium, the one drug tailor-made for her disorder, she refuses to remain on the treatment course — in part through a continuing denial, in part through a nostalgia for her exhilarating manic flights, in part for fear that if lithium should somehow fail her, she will know for sure that she is doomed.

Jamison's story has a reassuring, happy ending. Her professional career continues to be crowned with success — indeed, her reputation is made through the study of the very disorder that she and close family

members have inherited. She eventually accepts her illness and is able to control it with medication. She benefits as well from skilled psychotherapy. Most important from her point of view, the support of individuals who love her without restriction, and from colleagues who accept her despite her admitted disease, allows her to achieve peace. In a startling confession, she in fact admits that, if given the choice, she would choose manic-depressive disease over its absence — but only if the disease could be controlled by medication. Although she is occasionally nostalgic for the thrill of unalloyed mania, she concludes: "The current longings are, for the most part, only longings, and I do not feel compelled to re-create the intensities: the consequences are too awful, too final, and too damaging".

For lay readers, *An Unquiet Mind* may well become a classic, taking its place alongside such poignant memoirs as William Styron's account of depression, *Darkness Visible*, or M. A. Sechehaye's *Autobiography of a Schizophrenic Girl*. More scientific readers may be a bit disappointed, because Jamison skates only

lightly over the psychiatric literature, with the briefest discussions of diagnostic categories, the genetic basis of bipolar disease, and the neuropharmacology of treatment. I for one missed an analysis of the contributions — good and bad — of this peculiar disorder to the creative process. Who could be a more authoritative witness than Jamison, who is at once an eminent scientific researcher and a skilled, at times poetic, literary artist? And yet it is not fair to criticize Jamison on these accounts. This is not a book for the specialist; those of us who wish to probe more deeply need only look into Jamison's own accounts of creativity and madness in *Touched with Fire* (1993) or her well-regarded medical text *Manic Depressive Illness* (1990).

Nevertheless, this popular account speaks as well to those of us with a scientific or professional interest in manic depressive diseases.

In baring her medical soul, Jamison sets an example of courage; she knows that some will question her science and others her motives. She reminds us of how difficult it is for even the most respected professional to confront the possibility that he or she may be seriously ill and need proper treatment: not even those with supposedly special insights into the mind are spared. She gives an excellent account of the way in which an appropriate course of drugs, in combination with prompt and sensitive therapy, can be of far greater benefit than either could alone. Revealingly, she emphasizes the tremendous importance of that least quantifiable of entities — 'love'. Jamison tells us that she would not be here today were it not for the often remarkable and unquestioned love that she received from others; and, more disquietingly, she reveals how a lack of love, an unkind comment, even a disapproving look, can devastate an individual who feels vulnerable.

Jamison is not just writing about 'others': when she marshals the courage to tell her new supervisor about her illness, he has the grace to laugh and say: "If we got rid of all of the manic-depressives on the medical school faculty, not only would we have a much smaller faculty, it would also be a far more boring one". □

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The Three Ages and Death by Hans Baldung Grien. The picture is reproduced in *Aging: A Natural History* by Robert E. Ricklefs and Caleb E. Finch. The authors, leading workers in the physiological and evolutionary approaches to gerontology, provide an up-to-date and well-illustrated account of all aspects of the field that, like other volumes in the Scientific American Library series, will appeal to laypeople and specialists alike. W. H. Freeman/Scientific American Library, \$32.95, £19.95.



Star observer

Bernard Lovell

Edwin Hubble: Mariner of the Nebulae. By Gale E. Christianson. Farrar, Straus and Giroux: 1995. Pp. 420. \$27.50.

NOWADAYS it would be difficult to hold any discussion about the Universe without reference to the Hubble constant, the Hubble time, the Hubble flow or, indeed, the Hubble telescope. No doubt Edwin Hubble would be content that the space telescope bore his name, but whether he would be pleased that his measurements on the redshift of the galaxies implies a Big Bang origin for the Universe is a different matter.

In this scholarly book, Gale Christianson reveals that Hubble was never toler-

impressed by England — to such an extent that he asked for a postponement of the Mount Wilson appointment so that he could apply for a commission in the US Officers Reserve Corps. In September 1918, he sailed to Europe as Major Hubble, and when he eventually arrived at Mount Wilson in 1919 the 100-inch telescope had just been commissioned.

At that time, Harlow Shapley and Adriaan van Maanen were dominant figures at Mount Wilson. Both had achieved important results with the 60-inch telescope and both were destined to haunt Hubble's career. From measurements of the globular clusters, Shapley had devised a model of the Milky Way in which the Sun was far removed from the central regions, and he maintained that the spiral nebulae were objects within the Milky Way. This belief was supported by van Maanen's measurements of the transverse motions in the nebulae which, if they had been external to Shapley's Milky Way, would imply velocities far exceeding the velocity of light. In the two historic nights of 4 and 5 October 1923, Hubble recognized the Cepheid variable stars in the M31 spiral, and their period-luminosity curves left no room for doubt that the spiral was, indeed, an "island universe" lying far beyond the boundaries of Shapley's Milky Way.

It is fortunate that Shapley soon departed to Harvard, because he refused to believe the evidence for the Cepheids in M31. The depth of the rift between Hubble and Shapley is revealed by Christianson's references to Milton Humason, Hubble's faithful assistant. Humason maintained that before Shapley left Mount Wilson, he had shown him a plate of M31 on which he had marked the positions of Cepheids. Shapley refused to believe that they were Cepheids in M31, "then calmly took out his handkerchief, turned the plate over, and wiped them clean of Humason's marking".

With Humason's help, Hubble soon accumulated the data that established the linear relationship between redshift and distance of the nebulae (he always refused to use the word galaxies). In his 1929 and 1931 papers, Hubble published the velocity-distance relationship to 100 million light years, implying velocities of $20,000 \text{ km s}^{-1}$, but then, and ever afterwards, he was never convinced that these velocities implied an expansion of the Universe.

Initially, Hubble had good reason for this caution because the extrapolation to earlier cosmic times implied a timescale for the Universe less than the age of the Earth. With Hubble absent during the Second World War (he was at Maryland's Aberdeen Proving Grounds as head of external ballistics), Walter Baade was able to use the 100-inch telescope under black-out conditions. He discovered that there were two populations of the Cepheid variables and that a calibration error implied

that the Hubble distances and timescales had been underestimated by a factor of two. Although this removed the apparent conflict between the age of the Universe and that of the Earth, Hubble still would not concede that his measurements implied the large-scale expansion of the Universe.

Christianson gives an accurate account of Hubble's astronomical researches and of the lifestyle that formed the background to the astronomy. After the First World War, Hubble arrived at Mount Wilson as Major Hubble and to many, and particularly to his assistant Humason, he was always "the Major". He was an autocrat among scientists, insistent on formality and etiquette on the mountain and taking the liberty of making frequent and prolonged visits to England with his wife, Grace. These absences caused so much annoyance to Walter Adams, the director of the observatory, and to Vannevar Bush, president of the Carnegie Institution, that Hubble was not appointed as Adams's successor in 1945.

Hubble was stunned by the news that Ira S. Bowen was to be the new director, but the decision was a realistic one. The 200-inch telescope on Palomar Mountain was nearing completion and there is little evidence that Hubble would have been satisfactory as the director of the observatories. He was essentially an observational astronomer and, moreover, an astronomer with a single passionate interest in the nature and distribution of the nebulae. In 1949, in his first observations with the 200-inch telescope, he extended the redshift-distance relationship to a billion light years, but he remained unconvinced that his work had significance for any cosmological theory of the origin of the Universe. Christianson quotes from Hubble's manuscript notes in the Huntingdon Library about these observations that "there was no convincing evidence either of the expansion or non-expansion of the Universe".

Christianson's book is an important biographical work based on thorough searches of original material. In particular, the hundreds of pages of Grace Hubble's journals have revealed the extent of the Hubbles' social connections in England and the United States. The Hubbles relished the friendships that developed as Edwin's fame increased and, indeed, the book can be read with pleasure for its many vignettes on notable people of the time. Their life in the late 1930s is epitomized by the remark by Grace, driving home in the moonlight after dining with Charlie Chaplin and his then wife, Paulette Goddard: "Now whom do we want to meet?" □

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Edwin Hubble (1924).

ant of discussion about cosmological models. He was one of the great observers in the history of astronomy but thought that discussion of the cosmological implication of his results led to "a quagmire to be avoided at all costs". V. M. Slipher discovered the redshifts in the spectral lines of the spiral nebulae; and in October 1914 Hubble first learnt of these measurements which were to raise in a dramatic manner the question of the location of the spiral nebulae. At that time, Hubble was a student at Yerkes Observatory. He impressed the director and was recommended for a post at the Mount Wilson Observatory.

Hubble had recently returned to the United States from Oxford where he had studied jurisprudence as a Rhodes scholar. Christianson's well documented account of these years describes the influences that so greatly dominated Hubble's subsequent career. Hubble was deeply

Mistaken identities

James V. Neel

Human Biodiversity: Genes, Race, and History. By Jonathan Marks. *Aldine de Gruyter*: 1995. Pp. 321. DM96, \$46.95 (hbk); DM46, \$23.95 (pbk).

ANTHROPOLOGISTS and geneticists alike will find this well-researched and well-written book on human variation both instructive and challenging. And anyone interested in the concept of race, how it has been used and abused and how it is viewed today, will find it a fascinating read.

The first half is essentially didactic. The author begins by tracing the evolution of views of humankind, arguing that, just as Darwinian thought dominated the late nineteenth century, so Franz Boas dominated the early twentieth:

Boas brought cultural theory to its logical culmination in the 20th century. Darwin had undermined the biology of anthropocentrism and made it no longer possible to assert that the human species is "better" than a species of mole, for they are simply divergent offshoots of a common ancestor. So, too, Boas destroyed the underpinnings of ethnocentrism by which Western society saw itself as superior to other lifeways — it was different all right, but value judgments were ultimately based on arbitrary criteria.

Marks provides a brief but useful review of Mendelian inheritance and the mechanisms by which the human gene pool can change, as well as a summary of what we know about human evolution. He then looks at the efforts of physical anthropologists to classify humankind, the most recent low-water mark, in his opinion, being Carleton Coon's now discredited attempt in 1962 to recognize five races, and at the efforts of scientists to disentangle cultural and biological differences between groups of people. And inevitably there is a chapter on eugenics: as always, the recitation of the bigotry and prejudice of established biomedical scientists and the social actions stemming from the movement just 60 or so years ago make sobering reading.

Marks seeks to draw a firm distinction between racial anthropology, an objective study of biological differences among human groups, and racist anthropology, which superimposes value judgements on the observed differences. He examines the ways in which human variation can be studied, ranging from the traditional morphological approaches to the more recent biochemical (blood types, enzyme variants) and, now, molecular (DNA) classifications. He records the shock waves in the community of physical anthropologists when it became clear through the work of Boas, Harry Shapiro,



Barambo mu dancers from Poko in the Belgian Congo, from a hand-coloured slide by Herbert Lang (1913). The picture is taken from *American Museum of Natural History: 125 Years of Expedition and Discovery* by Lyle Rexer and Rachel Klein, which chronicles the history of the museum's expeditions around the globe, including those of the polar explorer Robert Peary and the anthropologists Franz Boas and Margaret Mead. Abrams, \$49.50.

Frederick Hulse and others that the cephalic index, that standby of physical anthropology, was significantly different in immigrant populations than that found in the native population, a shock that sent anthropologists racing to embrace the use of genetic markers.

The second half of the book is devoted to more-or-less self-contained essays on particular topics coming under the umbrella of 'human biodiversity'. Here the author works without fear or favour, studding his essays with trenchant comments. Having considered human diversity in the light of modern genetics, he sums up thus: "Like morphological differences across the human species, these (genetic) groupings are generally obvious when extremes are contrasted, but otherwise there is little in the way of reliable biological history to be inferred by genetics". And writing about the Human Genome Project, the first example of Big Biology, he says:

By virtue of focusing strictly on pathologies, the Human Genome Project, as originally proposed, falls into precisely the same conceptual framework held by the eugenicists. This is the idea that there is a single normal state for a given phenotype, whose nature is self-evident, and against which any deviation must be judged. This is true only to the narrowest extent, in the study of medical pathology. Where the eugenicists fairly explicitly held all deviation (including social and moral) from a narrowly defined ideal to be genetically

pathological, the assumptions of contemporary genome enthusiasts are instead more implicit in simply adopting the paradigm of medical pathology to molecular research.

He also attacks the Human Genome Diversity Project (which came into being partly as a response to such criticism):

The major goal in this effort, unfortunately, is thus also guided by an archaic idea: the establishment of the ultimate genetic phylogeny of human groups. In pursuit of this objective, advocates are obliged to maintain that non-European human populations are generally "pure," and have been spared the vagaries of history, of contact, and of gene flow — assumptions that are certainly gratuitous.

These are strong words, apparently strongly motivated by the concern that the intermingling of early human populations, like those recorded for historical populations, were so extensive as to preclude the possibility of reconstructing the evolutionary relationship of human populations.

There is, perhaps, in this broadside the danger of throwing the baby out with the bathwater. Dendrograms are also useful graphic representatives of the similarities and differences between populations — however they are arrived at. But it must always be remembered that custom requires a 'best' dendrogram to be presented, and other dendrograms of the relationship of a set of populations, almost



Kwakiutl North American Indian mask of "born-to-be-head-of-the-world" collected by George Hunt, Franz Boas's field assistant, in 1901. From *American Museum of Natural History* (see p.589).

as satisfactory by the 'minimum string' concept, may look quite different. I agree wholeheartedly though with the author's statement that:

The a priori knowledge that most human genetic variation is polymorphic, rather than polytypic, should make it more important to preserve many samples from relatively fewer groups, than to preserve few samples from many groups, if one wishes to study the general extent and nature of human genetic diversity.

In dealing with the adaptive nature of human variation and the 'health' differences between populations, Marks unsurprisingly concludes that although such human traits as bipedal locomotion, large cranial capacity and pelvic structure are adaptive, the adaptive significance of most within-human variation is obscure, apart from such examples as skin pigmentation and the sickle-cell trait. Furthermore, ethnic differences in disease patterns are not related to genetic variation, again with a few exceptions such as malaria resistance due to the possession of the sickle-cell trait.

Marks also returns to the hoary question of "Human Traits: Heritage or Habitus?", where heritage is defined as inborn (that is, genetic) and habitus as acquired (that is, culturally imposed). Dismissing all comparisons of the behaviour of humans with that of non-primates, he writes:

When we confine ourselves to our close relatives, however, we find (1) such a difference between human behavior and that of the apes, and (2) such a diversity of behaviors among the apes that it becomes difficult to argue for much of any human behavior being the result of heritage rather than of habitus.

Although granting that "human behavior, like all phenotypes, has a genetic component", Marks then develops the case that "it is difficult, if not impossible, to match genes to behaviors". Geneticists interested in the genetic basis of deviant behaviour will find his discussion stimulating.

Marks ends by reiterating a dominant theme of the book, that scientists dealing with human differences have social responsibilities not shared by those dealing with differences between clams or fruitflies, responsibilities too often abused in the past. Nor is he too happy with the present: "We seek a path of self-awareness through genetics, yet we are constantly led into intellectual cul-de-sacs". The overarching theme of this book is that despite the obvious differences between individuals and groups of individuals, the similarities are now seen as so great, and the gradations between groups so subtle, that attempts at ethnic classification are almost reduced to a trivial pursuit. But as he says in his concluding sentence, "it is tempting to commit those mistakes again and again". This book will be much discussed. Read it. □

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Current revolutions

Andrew Holmes-Siedle

The Quantum Dot: A Journey into the Future of Microelectronics. By Richard Turton. W. H. Freeman/Oxford University Press: 1995. Pp. 211. £11.99 (pbk), \$25 (hbk).

GIVEN the need for information technology to progress continually, what is the next step when we have exhausted the possibilities of microelectronic devices on silicon? One answer is quantum dots, wells and wires. Although the author has chosen a catchy, futuristic title alluding to these possibilities, he deals with them only in the second half of the book.

Quantum dots are minute microelectronic structures usually made of III-V compounds such as gallium arsenide. The scale of the structure, about 10 nm, is small enough to allow the wave properties of the electron to come into play. Using the growth of films of III-V compounds by molecular-beam epitaxy, linked with sub-micrometre lithography, such 'droplets' can be produced in tightly packed arrays, and 'quantum wells' can be made inside. The well is like a little box (or conduit), suitable for handling a single electron. These III-V compounds also convert current to light, so optoelectronic methods can

be explored. Very small packets of charge can be piped around these structures — smaller packets than are possible for silicon. Here lies one of the possible waves of the future. AT&T is exploring it, so there must be something in it.

This is primarily a book on integrated circuits, however, firmly footed in the silicon age. Intended for the uninitiated reader, it outlines the basic principles of how countless functions are packed into a small 'chip' — mostly using device physics from before the age of quantum wells — and ends with a useful explanatory description of the possible next steps after silicon.

Whenever I explained to my late mother-in-law the design of a silicon chip, she usually responded by asking: "How ever do you think of these things?" Modestly, I would reply by saying that it was not me alone who did the thinking, that I had been trained to and so on. But the question has resonance. Although not the main purpose of the book, one of its merits is that it gives a hint of just how we do think of these things. With unusual thoroughness — one might say patience — the author explains the physical effects used in the design of semiconductor devices in terms of simple concepts, drawing analogies to tennis balls and pieces of string. One could say that he has reduced semiconductor physics to childish concepts. This is not intended as a sneer; while looking at the pictures, it occurred to me that, for some of the best scientists I know, the process of clarifying physical observations consists of reduction to very childish concepts. It is the drive to their thinking; only later comes complexity, when the concepts are subjected to the trappings of conventional disciplines such as those of mathematics or computer software.

At least the author avoids talking about 'silicon chips'. Long gone are the days when device designers, like woodsmen, hacked a piece of semiconductor off a crumbly ingot. Chips are crafted by large expensive machines, and ingots are six feet long. But the mechanics of production is only lightly touched on here. The author concentrates on the physics of the products.

It is a pity that he did not run the manuscript past an electronic engineer: the description of the basic MOS gate logic circuit is completely wrong. But the physics is sound, and, more to the point, can be understood by the lay reader. The book's occasional factual defects should not prevent it from encouraging readers to explore further how we may control and channel electrons and holes in electronic gadgets. Its usefulness need not stop at the lay person; an intuitive scientist will also get a charge out of it. □

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Cytokine receptor signalling

James N. Ihle

Many cell functions are regulated by members of the cytokine receptor superfamily. Signalling by these receptors depends upon their association with Janus kinases (JAKs), which couple ligand binding to tyrosine phosphorylation of signalling proteins recruited to the receptor complex. Among these are the signal transducers and activators of transcription (STATs), a family of transcription factors that contribute to the diversity of cytokine responses.

NUMEROUS aspects of lymphoid and myeloid cell function are controlled by a group of ligands termed cytokines, all of which signal through a related set of receptors. The conserved extracellular motifs of these receptors¹ are apparently derived from the fibronectin type III module. Different family members contain one to three chains, one or more of which generally have limited similarity in the membrane-proximal region (often referred to as box1/box2 motifs). All such receptors are associated with one or more members of a once obscure family termed the *Janus* kinases (JAKs). These kinases couple ligand binding to tyrosine phosphorylation both of various known signalling proteins and of a unique family of transcription factors termed the signal transducers and activators of transcription (STATs).

JAKs in cytokine signalling

The tyrosine kinases Fes, Lck, Hck, Lyn, Fyn and Syk are all activated or phosphorylated in response to individual cytokines^{2,3}, and the activation of Src family kinases by interleukin (IL)-2 is well documented⁴. Src kinases are not activated by most cytokines, however, and many IL-2 responses do not require Src. Similarly, the other kinases mentioned have not been widely implicated in cytokine signalling. By contrast, activation of all known cytokine receptors induces the tyrosine phosphorylation and activation of one or more JAK kinases associated with the receptor, and JAK activation is required for most, if not all, receptor functions.

The JAK family consists of Jak1, Jak2, Jak3 and Tyk2 (refs 2, 5), each of relative molecular mass in the range 120–130K. All have a carboxy-terminal kinase domain immediately preceded by a pseudokinase domain. No other recognizable protein motifs are present, although amino-terminal domains are conserved in the different family members. With the exception of Jak3, which is primarily expressed in haematopoietic cells, JAKs are ubiquitously expressed. The *Drosophila hopscotch* locus encodes a JAK homologue⁶; mutations at this locus cause developmental abnormalities, and a gain-of-function mutation encoded by the *Tum^t* allele causes a form of leukaemia in flies^{7,8}.

There are three patterns by which JAK kinases associate with receptors, depending on the receptor structure (Fig. 1). Several cytokines use single-chain receptors which associate with Jak2 (or, to a variable and lesser extent, with Jak1) by means of their conserved membrane-proximal domain. Association can be constitutive or enhanced by ligand binding. Receptor aggregation induces concomitant aggregation of Jak2, which is thought to allow transphosphorylation of the KEYY site in the kinase activation loop, dramatically increasing catalytic activity. Activated Jak2 could then phosphorylate itself, the receptor and cellular substrates recruited to the receptor complex. As this model predicts, mutations in the extracellular domain of the receptor leading to ligand-independent receptor aggregation also render Jak2 activation constitutive^{9,10}.

The IL-3 and IL-6 cytokine families typify the second association pattern. The receptors for IL-3, IL-5 and granulocyte/macrophage-colony-stimulating factor (GM-CSF) each consist of a ligand-binding α -chain associated with a common β_c -chain¹¹. Jak2 associates with the membrane-proximal domain of the β_c -chain¹², aggregation of which mediates signal transduction. Similarly, the receptors for the cytokines IL-6, IL-11, CNTF, OSM and LIF all use ligand-specific binding chains that

associate either with gp130 or the related signalling protein LIFR- β ¹³. Unlike other receptor chains, gp130 associates with and activates Jak1, Jak2 and Tyk2 (refs 14, 15). Activation of Jak1 is essential for signalling and depends on the presence of either Jak2 or Tyk2 (ref. 16).

The IL-2 and interferon (IFN) receptor families typify the third association pattern, in which two chains are required for signalling. The IL-2 receptor consists of α -, β - and γ_c -chains³, the cytoplasmic domains of the last two being required for signal transduction. The γ_c -chain also forms part of the receptors for interleukins 4, 7, 9 and 15, each of which also contains a ligand-specific α -chain related to the IL-2 receptor β -chain (or, in the case of IL-15, both the β - and γ_c -chains¹⁷). In each case, Jak1 associates with the membrane-proximal region of the IL-2 receptor β -chain or the ligand-specific α -chains, while Jak3 associates only with the shared IL-2 receptor γ_c -chain. Ligand-induced receptor aggregation then brings Jak1 and Jak3 together.

Similarly, the receptors for IFN- α/β and IFN- γ each contain at least two chains required for signal transduction. In the IFN- α/β receptor, Jak1 associates with β -chain¹⁸ while Tyk2 associates with the α -chain¹⁹. In the IFN- γ receptor, R1 (the α -chain) associates with Jak1 (ref. 20) while R2 (the β -chain) associates with Jak2 (S. Pestka, manuscript submitted). Here, both JAKs are required for activation of either JAK². The IL-10 receptor consists of a cloned chain related to the IFN receptors and a second, uncloned chain²¹, which are thought to associate with Jak2 and Tyk2, the JAKs that are activated by IL-10.

In all cases, the primary function of the ligand is to mediate receptor aggregation and the concomitant homo- or heterotypic aggregation of JAKs. Curiously, only JAK2 is activated homotypically. Other JAKs may not autophosphorylate efficiently, as suggested by studies of Jak1 phosphorylation in IL-6 signalling, although any JAK autophosphorylates and becomes activated when overexpressed in insect cells (F. W. Quelle and J.N.I., unpublished observations). In some cases, the receptor structure may dictate the need for two JAKs, or both JAKs may be needed to form a high-affinity receptor complex²².

Several observations underline the critical role of JAKs in signalling. Genetic selection of cells unable to respond to IFN produces cell mutants lacking Jak1, Jak2 or Tyk2, and addition of the missing JAK restores signalling^{2,23}. Moreover, deletions or mutations affecting JAK association inactivate all receptor functions in all receptors examined. Deletions or mutations in the IL-2 receptor γ_c -chain that disrupt Jak3 association inactivate the receptor, producing X-linked severe combined immunodeficiency in humans¹⁷, and kinase-negative Jak2 is a dominant suppressor of the cellular response to erythropoietin (Epo)²⁴ or IL-6¹⁶. Lastly, the absence of Jak3 in mice or humans is associated with deficiencies in lymphoid development which result in severe combined immunodeficiencies^{87–90}.

JAKs and cytokine-induced signalling

Like the receptor tyrosine kinases, cytokine receptors activate many signalling pathways, generally by means of phosphotyrosine residues, which are recognized by SH2 domains on the signalling molecules (Fig. 2). Most cytokines activate Ras by inducing phosphorylation of the adaptor protein SHC²⁵. Other common targets include the p85 subunit of phosphatidylinositol

3-OH kinase (PI(3)K) and (less often) phospholipase C- γ 1 (PLC- γ 1), both of which (like SHC) are recruited to the receptor by virtue of their SH2 domain. Mutations disrupting JAK association with the receptor complex eliminate SHC and p85 phosphorylation^{26,27}, but whether JAKs directly phosphorylate SHC, PLC- γ 1 or p85 is still unclear.

The receptor domains required to recruit various signalling proteins and the role of the proteins recruited can be assessed by the effects of mutations in the receptor. SHC and p85 phosphorylation, together with Ras activation, require the membrane-distal domain^{26,28}, which is not required for mitogenesis. In haematopoietic cells, Ras activation is thought to suppress apoptosis²⁹ and activate the transcription factor NF-IL-6 (ref. 30), it may also contribute to STAT activation. The function of PI(3)-K is unknown, but it may contribute to preventing apoptosis³¹ and/or activate the Ras pathway by an SHC-independent mechanism^{32,33}.

Cytokines also induce tyrosine phosphorylation of Vav. Although Vav is not required for haematopoiesis³⁴, it is critical for signalling by the T- and B-cell antigen receptors in lymphoid cells³⁵⁻³⁷. With cytokine receptors, phosphorylation requires JAK activation³⁸ but only the membrane-proximal region of the receptor is needed, and Vav may be recruited to the receptor complex through phosphotyrosine residues on JAKs³⁹.

Among the cytokines, IL-4 is unusual in that it has no effect on Ras⁴⁰ and induces tyrosine phosphorylation of the protein insulin-receptor substrate-1 (IRS-1) or its relative IRS-2/4PS (ref. 41), which are thought to activate several signalling pathways⁴². These proteins associate with the IL-4 receptor α -chain, and mutations affecting this association eliminate a mitogenic response. Phosphorylation requires JAK activation⁴³, although it is unclear if IRS-1 or IRS-2 are JAK substrates. Growth hormone⁴⁴ and IL-9 (ref. 45) also induce phosphorylation of IRS-1 and IRS-2, respectively.

The role of Lck in signalling by the IL-2 receptor is still uncertain. Lck binds to the membrane-distal domain of the receptor's β -chain through its kinase domain⁴⁶, however, and deletions of this domain block IL-2 induced *c-fos* transcription. Syk, too, is constitutively associated with the membrane-proximal region of this chain⁴⁷. The ancillary kinases may therefore phosphorylate the receptor so as to allow the recruitment of signalling proteins, or phosphorylate proteins recruited to the receptor complex that are not substrates for JAKs.

Cytokine-induced STAT activation

Cytokines also activate the STAT class of transcription factors. STATs were first identified in studies of IFN-regulated gene expression²³, and Stats 1 and 2 are tyrosine-phosphorylated in response to IFN- α/β . Phosphorylation triggers formation of a complex with the DNA-binding protein p48, which moves to the nucleus and activates transcription of genes bearing the interferon-response element (ISRE). Similarly, Stat1 phosphorylation induced by IFN- γ causes it to dimerize⁴⁸, move to the nucleus and bind to the gamma-activated sequence (GAS) in IFN γ -responsive genes. These findings suggested that other cytokines might also use STATs, a hypothesis which was rapidly borne out by the cloning of the genes for Stats 3 (refs 49, 50), 4 (refs 51, 52), 5 (ref. 53) and 6 (refs 43, 54). Indeed, virtually all cytokines appear to activate one or more STATs.

The STATs contain a carboxy-terminal SH2 domain, an SH3-like domain and several conserved amino-terminal regions, in addition to a conserved region in the middle of the protein that binds DNA⁵⁵. Tyrosine phosphorylation of a carboxy-terminal site mediates homo- or heterodimerization through the SH2 domains, triggering movement to the nucleus and DNA binding. Serine/threonine phosphorylation, possible mediated by the MAP kinases^{56,57}, also plays a role in activation, linking STAT and Ras activation.

STATs recognize related elements consisting of the inverted repeat TTNCNNNA. Binding and amplification reactions with random oligonucleotides show that the 'optimal' binding site for Stats 1 and 3 is TTCC(C/G)GGAA⁵⁵, but preferred binding sites for each STAT can readily be identified among known GAS sites by competition approaches⁵⁸. The ability of various STATs to heterodimerize may also influence their sequence specificity.

In all cases examined, mutations in cytokine receptors that eliminate their association with JAKs abolish STAT phosphorylation, and JAKs can perform the single tyrosine phosphorylation necessary to activate DNA binding by STATs *in vitro*⁵⁹. Individual JAKs exhibit no detectable preference for specific STATs *in vitro*, however, and their remarkable specificity *in vivo* does not reflect kinase specificity.

Cytokine specificity and redundancy

Cytokines typically induce both overlapping and unique biological responses, which reflect the different signalling pathways

FIG. 1 Models for the association and activation of JAKs with and by different cytokine receptors. The first class of receptors (top) consists of single chains which primarily associate with Jak2 through a receptor membrane-proximal domain containing the box1/box2 motif (see also Fig. 2). The second class of receptors consists of two chains. One chain is required for ligand binding and a second shared chain is required for signalling. The shared signalling chain associates with one or more of the JAKs, as indicated, through a membrane-proximal domain containing the box1/box2 motif. The third class of receptors contain at least two chains, two of which are required for signalling. In these cases, JAKs associate with both signalling chains. In the IL-2 receptor subfamily, the shared γ -chain associates with Jak3 while the IL-2 receptor β -chain, or corresponding α -chains, associates with Jak1 through the membrane-proximal domain containing the box1/box2 motif. Among the IFN receptors, the individual chains associate with the JAKs indicated. In all cases, ligand-induced receptor aggregation is proposed to aggregate the associated Jaks and allow their activation by transphosphorylation. The JAKs are indicated in yellow.

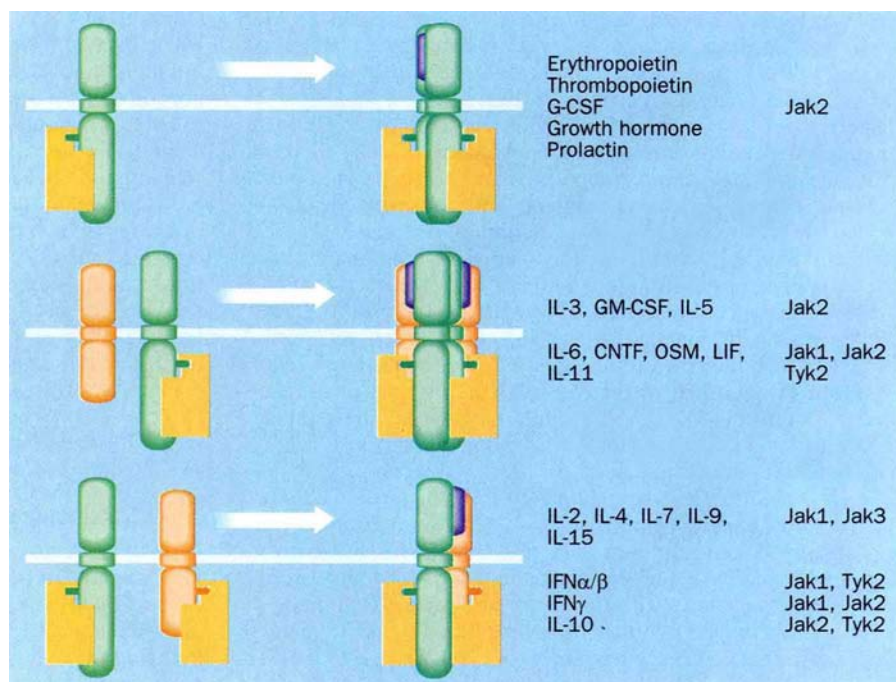
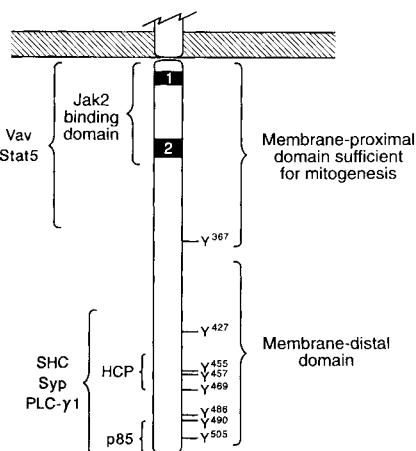


FIG. 2 Signalling proteins associating with the erythropoietin receptor. The Epo receptor associates with and activates Jak2. Jak2 association occurs through the membrane-proximal region in the box1/box2-containing domain. In response to Epo, tyrosine phosphorylation of the receptor occurs at sites in the membrane-distal region, together with phosphorylation of Vav, Stat5, SHC, PLC- γ 1, Syp and p85. The membrane-proximal region of the receptor is necessary and sufficient for Vav and Stat5 phosphorylation as well as for a mitogenic response. The membrane-distal region of the receptor, which is dispensable for mitogenesis, is required for the recruitment and subsequent phosphorylation of SHC, Syp, PLC- γ 1 and p85. Tyrosine phosphorylation of the distal region of the receptor also creates binding sites for HCP and recruits the phosphatase to the receptor complex in which it negatively influences receptor activity.



involved. In T cells, for example, IL-2, 4 and 9, all of which utilize the IL-2 receptor γ -chain, activate Jak1 and Jak3, whereas IL-10 and 12 activate Jak1 and Tyk2 and Jak2 and Tyk2, respectively⁹¹⁻⁹³. The STATs show an even greater range of responses, each cytokine activating a unique set. Different cytokines may also activate Ras, IRS proteins or other cytoplasmic kinases. Some of the differences in the effects of these cytokines⁶⁰ will certainly reflect the constellation of signalling pathways they activate, and mutants that uncouple activation of the individual pathways will be needed to dissect them.

Several cytokines have remarkably similar biological activities, partly because their receptors contain common signalling chains. Some of the similarities, however, reflect the STATs involved. G-CSF, for example, shares many properties with IL-6 when examined on the same cells, largely because both activate Stat3.

The specificity with which STATs and other proteins are activated relies on the specificity with which they are recruited to the receptor complex. Often this depends upon recognition of phosphotyrosine docking sites on the receptor by the STAT SH2 domain^{43,61}. Docking sites exist on the β -chain of the IL-2 receptor and the α -chains of the IL-4 and IL-10 receptors for Stats 5, 6 and 3 respectively (refs 43, 62) and R. D. Schreiber, submitted). Indeed, addition of relatively short peptides containing a docking site to a receptor allows Stat recruitment and activation⁶¹, and switching STAT SH2 domains produces a corresponding change in the receptors required to activate them⁶³.

Other receptors, such as those for growth hormones⁶⁴ can recruit and activate STATs without being phosphorylated, either by means of an alternative type of association, or by means of an undiscovered receptor chain. The potential complexity of STAT recruitment is illustrated⁶⁵ by Stat2's role in recruiting and phosphorylating Stat1 in response to IFN- α/β . In this case, Stat2 is thought to provide docking sites for Stat1 after first binding to the receptor complex and being phosphorylated itself, indicating that Stat1/Stat2 heterodimerization may be important.

Biological functions of the STATs

STATs regulate the expression of a wide range of genes. Transcription of many genes controlled by IFN²³, some of which are involved in antiviral responses, requires Stat2 and/or Stat1. Similarly, IL-6-induced expression of the acute-phase response genes requires Stat3 (ref. 13), whereas Stat5 mediates prolactin-induced transcription of several proteins secreted in milk⁵³. Stat6 binds to an element in the immunoglobulin locus required for

IL-4-induced class switching^{66,67}, and mediates IL-4-induced upregulation of the major histocompatibility complex (MHC) class II antigen, various immunoglobulin receptors and other cell surface proteins.

Although the STATs are ubiquitously expressed (with the exception of Stat4; ref. 51), they probably regulate different genes in different cell types. Stat5 induces transcription of the β -casein and whey acidic protein (WAP) genes in mammary gland cells, for example, but not in myeloid cells. Like many transcription factors, STATs probably act as components of multi-protein transcriptional complexes, which may also contain cell lineage- or differentiation-specific components. Stat5-induced transcription of the WAP gene also requires a binding site for nuclear factor 1 (ref. 68).

STATs appear only to be involved in functional (as opposed to mitogenic) responses to cytokines, as IFNs do not generally induce proliferation and, indeed, are typically antiproliferative⁶⁹. Moreover, IL-6 (ref. 61), IL-2 (ref. 62) and IL-4 (ref. 43) mutant receptors can trigger cell proliferation without activating Stats 3, 5 and 6 respectively.

Phosphatase regulation of cytokine signalling

Cytokine receptors are also negatively regulated, in part by haematopoietic cell phosphatase (HCP, also termed PTP-1C or SH2-PTP1). HCP contains a carboxy-terminal catalytic domain specific for tyrosine and two amino-terminal SH2 domains, the first of which facilitates recruitment of HCP to activated receptors, including *c-kit*⁷⁰, the IL-3 receptor β -chain⁷¹, the B-cell antigen receptor⁷² and the Epo receptor^{73,74}. The mouse mutant *motheaten*, which lacks functional HCP, suffers from numerous haematopoietic abnormalities attributable to cell overproliferation^{75,76}, and over- or underexpression of HCP suppresses or enhances cytokine responses⁷¹. In some cell lines, recruitment of HCP to the receptor complex is associated with dephosphorylation of Jak2 (ref. 74), suggesting that JAKs are critical substrates.

By contrast, the related phosphatase Syp (also termed SH-PTP2 or PTP-1D) is thought to stimulate signalling. Syp too contains two SH2 domains which allow it to bind to the receptors for IL-6 and other cytokines⁶¹. It is then tyrosine-phosphorylated, although it is unclear whether a JAK is responsible. The result is to activate MAP kinase, perhaps by binding Grb2 and activating the Ras pathway⁷⁷. It may also activate Src-family kinases by dephosphorylating their carboxy-terminal phosphotyrosines.

As already noted, the specificity of signalling generally depends on the receptor complex and not the JAKs. Indeed, overexpression of JAKs promiscuously activates endogenous JAKs and STATs. To retain specificity, activated JAKs that dissociate from the complex must thus be inactivated, perhaps by cytoplasmic phosphatases such as HCP.

Other receptor systems

Although they are best known for their role in cytokine-receptor signalling, JAKs and STATs also participate in signalling by other receptors. EGF-induced *c-fos* expression requires STATs,

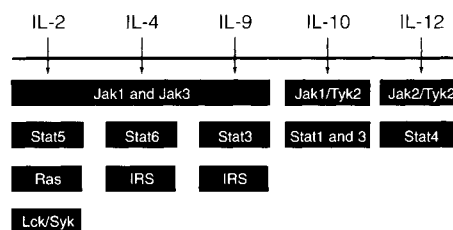


FIG. 3 Diversity in cytokine-induced T-cell signalling. The spectrum of JAKs and signalling proteins activated by the individual cytokines is indicated. No information is available regarding the activation of the Ras pathway or IRS-2 phosphorylation by IL-10 or IL-12. Similarly, IL-4, IL-9, IL-10 and IL-12 have not been reported to activate other cytoplasmic kinases.

which are thought to be recruited to a *cis*-inducible element in the promoter^{49,78-80} following SH2-mediated interactions with the phosphorylated EGF receptor, and subsequent tyrosine phosphorylation. The purified EGF receptor can indeed directly phosphorylate Y701 of Stat1 (ref. 59), and it will be important to determine which other tyrosine kinases share this capacity.

JAKs and STATs are also activated by angiotensin II, whose receptor is a member of the G-protein-coupled receptor family^{81,82}. Several such receptors trigger tyrosine phosphorylation of focal adhesion kinase⁸³ or Src kinases⁸⁴ on binding ligand, and it will be interesting to determine whether all members of this family activate JAKs and STATs.

Cellular transformation

JAKs and/or STATs may also contribute to cellular transformation. As already mentioned JAK mutations in *Drosophila* can cause transformation^{7,8}, and mutations leading to constitutive dimerization of cytokine receptors (and thus JAK activation) may cause transformation. Moreover, HTLV-1 infection is thought to lead, whether directly or indirectly, to a similar receptor-mediated activation of the pathway⁸⁵. So far, however, JAKs have not been shown to transform mammalian cells.

The observation that Stat3 is constitutively phosphorylated in *v-src*-transformed cells has also been taken to support a role for STATs in transformation⁸⁶. *v-Src* phosphorylates a variety of cellular substrates, however, only some of which are involved in transformation, and as noted above, there is no other evidence that Stat3 is involved in mitogenic responses.

Future directions

Although JAKs play a critical role in cytokine-induced signalling, relatively little is known about their structural domains. JAKs are phosphorylated at several unidentified sites in response to cytokines in addition to the activation loop, which are probably important in recruiting additional signalling proteins to the receptor complex. Moreover, the domains that associate with the membrane-proximal regions of the receptors have yet to be identified, and the functions of the JAK-homology domains and the pseudokinase domain are unknown.

Other properties of the STATs also remain unclear. Of particular interest is the mechanism by which STAT dimers translocate to the nucleus; is it passive or facilitated? In the IFN α / β -induced transcription complex, p48 is essential. This protein is a member of a family that includes IRF-1 and ICSBP: do these proteins functionally associate with other members of the STAT family? Other questions, such as the biological role of each JAK and STAT, will be resolved as mice lacking functional genes for the different JAKs and STATs (or combinations thereof) become available.

Finally, most cytokines induce cell-cycle progression, which is generally assumed to require transcriptional activation of *c-myc*. The involvement of several signalling pathways in these responses, including the Ras pathway, has been ruled out by the use of receptor mutants, and the identity of the pathways involved will be of great interest. The central role of JAKs and STATs in the cellular responses controlling numerous aspects of lymphoid and myeloid function also makes them attractive targets for drug development. □

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Hydrous, silica-rich melts in the sub-arc mantle and their relationship with erupted arc lavas

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Hydrous, silica-rich melts migrating through the mantle are preserved as glass inclusions in mantle minerals in xenoliths from Philippine arc lavas. These melts, with chemistries that indicate an origin by very low degrees of melting of the subducted ocean crust, have altered their host peridotites, yielding a metasomatized mantle. This 'fertilized' mantle is the source region of the arc magmas, which share continuous chemical trends with the melt inclusions, reflecting mixing and/or varying degrees of melting. These observations provide direct evidence for the importance of slab-mantle interactions in the genesis of island-arc magmas.

MIGRATING aqueous fluids or melts derived from subducting lithosphere are thought to enrich the overlying mantle 'wedge' in water and many trace elements. This metasomatized mantle region becomes the source of island-arc basalts¹, and the trace-element characteristics (high concentrations of large-ion lithophile elements (LILE) and low concentrations of high-field-strength elements (HFSE)) of the arc volcanic rocks reflect the metasomatic process (see, for example, ref. 2).

Much of the evidence for this model for the origin of island-arc basalts has been derived indirectly through the systematic study of the composition of the end products, the volcanic rocks^{2,3}, and there have only been a few direct studies of the metasomatism of the mantle rocks from these regions^{4,5}. Therefore, important aspects of the metasomatic model have remained somewhat hypothetical. In particular, it has not been clear whether the primary metasomatizing phase is a silicate melt or an aqueous fluid.

We have identified the remnants of melts migrating through the mantle wedge beneath island arcs, preserved as glass inclusions trapped within minerals in metasomatized mantle xenoliths in a calc-alkaline arc-front volcano (Batan island, Luzon Arc, Philippines). The xenoliths are porphyroclastic harzburgites containing late hydrous metasomatic minerals. Glass inclusions occur in both primary and metasomatic minerals. They show hydrous, silica-rich compositions, and their trace-element signature is characterized by enrichment in very incompatible elements, with negative Nb and positive Pb anomalies. Moreover, the glass inclusions have very low heavy rare-earth element (HREE) and Y concentrations, and correspondingly high La/Yb and Sr/Y ratios. These features and the entrapment temperatures (~920 °C) of the inclusions are consistent with experimental results obtained from partial melting of metabasalts, indicating that the trapped melts were formed by melting in the subducted oceanic crust. These slab-derived melts metasomatized the depleted spinel harzburgites through the recrystallization of the primary paragenesis to form iron-enriched olivine and orthopyroxene and the crystallization of hydrous minerals. The melts also impart their own geochemical fingerprint, as shown by the trace-element composition of olivine separates, which is dominated by the inclusions. We further show chemical correlations

between the inclusions and the host andesites, indicating that the chemical characteristics of the erupted mantle-derived andesitic basalts are closely related to these metasomatic melts migrating through the sub-arc mantle.

Xenolith and glass inclusion description

The island of Batan belongs to the north Luzon volcanic arc, where two major episodes of volcanism have been recognized. An early phase of volcanism (late Eocene to Miocene) is associated with subduction of the Indian Ocean plate beneath the western rim of the Philippine plate. Post-15 Myr BP (before present) volcanism (which includes Batan island volcanism) is associated with subduction along the Manila trench of the Eurasian plate^{6,7}. Mantle xenoliths occur within potassic calc-alkaline basaltic andesites from Mount Iraya (<0.1 Myr)⁸, the youngest volcano on Batan.

The xenoliths are mainly porphyroclastic spinel-bearing harzburgites containing olivine (Fo₉₀₋₉₃), orthopyroxene (En₉₀₋₉₂) and spinel (Cr# (100 Cr/(Cr + Al + Fe³⁺)) = 53–67). They are characterized by LILE (Ba, Rb, Pb, K and U) enrichment and negative anomalies in HFSE (Ti, Zr, Hf, Nb and Ta)^{4,5} (Fig. 1a). ⁸⁷Sr/⁸⁶Sr, ¹⁴³Nd/¹⁴⁴Nd and δ¹⁸O values of the xenoliths are similar to those of the host lavas^{5,9}. Thus, although the xenoliths come from the upper part of the mantle wedge, their isotopic characteristics indicate that they have been affected by the same metasomatic fluid or melt as the source of the host lavas. A metasomatic episode is recorded in some nodules by recrystallization of primary olivine and orthopyroxene porphyroclasts into polygonal iron-enriched olivine and orthopyroxene neoblasts (Fo₈₀₋₈₆ and En₈₆₋₈₈) with development of mosaic textures, and by the crystallization of undeformed hydrous minerals (phlogopite, pargasite and hornblende)¹⁰.

Numerous glass inclusions occur in porphyroclasts and metasomatic minerals. In olivine and orthopyroxene porphyroclasts, rounded inclusions (size <40 µm) occur as secondary trails along fissures, whereas in metasomatic minerals (iron-enriched olivine, orthopyroxene and hydrous minerals) the inclusions are isolated (diameter between 30 and 150 µm) with negative crystal shapes imposed by the host mineral, and they are randomly scattered throughout the host mineral or more rarely outline

zones of crystal growth. Such features are typical of primary inclusions containing parental liquids which have crystallized the host minerals¹¹. The inclusions generally contain quenched glass and one shrinkage gas bubble, but some contain a combination of 'daughter' crystals grown from the trapped melt. Phlogopite, amphibole (pargasite and hornblende), orthopyroxene and clinopyroxene have been analysed inside silicate inclusions in olivine (Table 1). This association is similar to the metasomatic paragenesis occurring in the xenoliths. Heating-stage experi-

ments were performed with a high-temperature optical apparatus on some primary glass inclusions trapped in olivine neoblasts. Final homogenization temperatures were close to $920 \pm 10^\circ\text{C}$, corresponding to the entrapment temperature of the inclusions during host mineral growth¹¹. The occurrence of composite inclusions consisting of silicate glass + H_2O (vap + liq) and silicate glass + sulphide suggests the former existence of a homogeneous ($\text{H}_2\text{O} + \text{S}$)-rich silicate melt phase (or supercritical silica-rich fluid phase). The presence of primary H_2O -fluid inclusions (size $< 100\ \mu\text{m}$) and spherical sulphide droplets (size $< 50\ \mu\text{m}$) in the metasomatic minerals indicates water and sulphur saturation of the trapped melt during the entire course of its crystallization.

The textural and petrographic features of the Batan xenoliths record the following sequence of events (see Fig. 2). (1) Primary

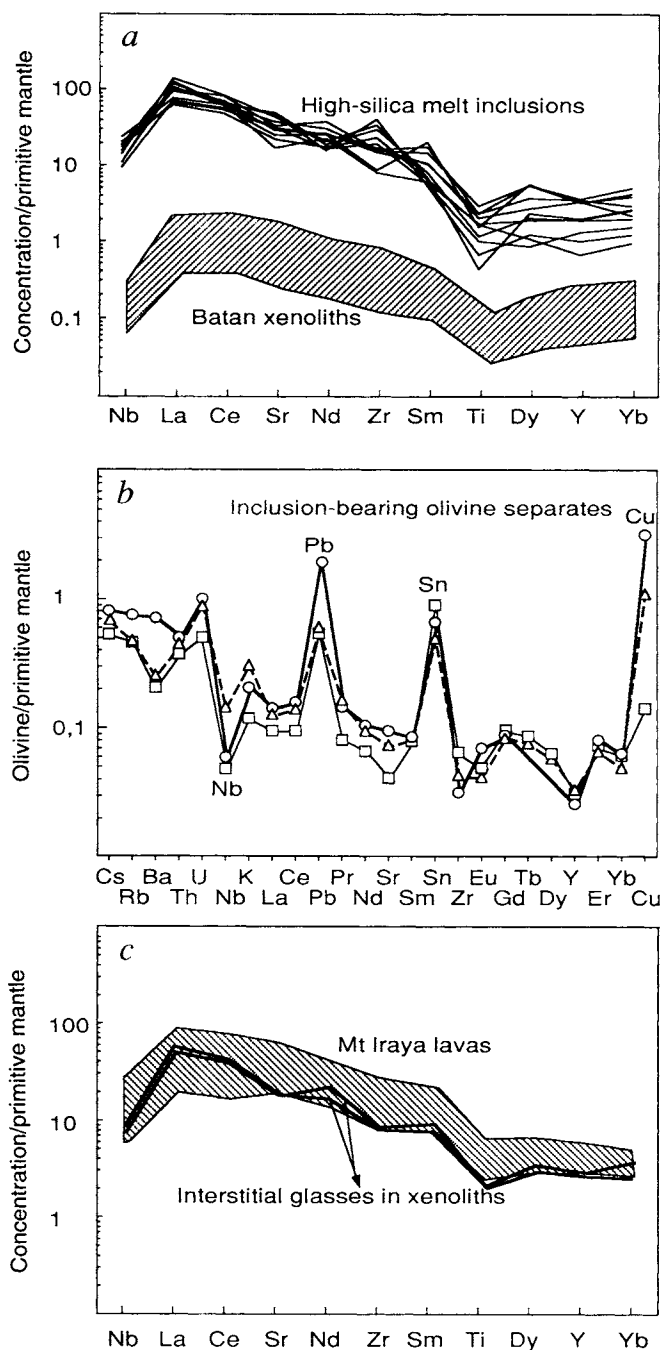


FIG. 1 Trace-element compositions for a, glass inclusions trapped in olivine crystals from Batan xenoliths analysed by ion microprobe (the shaded area delineates the range of bulk compositions of Batan xenoliths⁴), b, inclusion-bearing olivine separates analysed by spark source mass spectrometry, and c, representative interstitial glasses found in xenoliths (ion probe data) and Batan lavas (restricted to Mount Iraya andesites^{10,23}). Elements are arranged by relative compatibility in oceanic basalts¹³ and normalized to primitive-mantle values¹³.

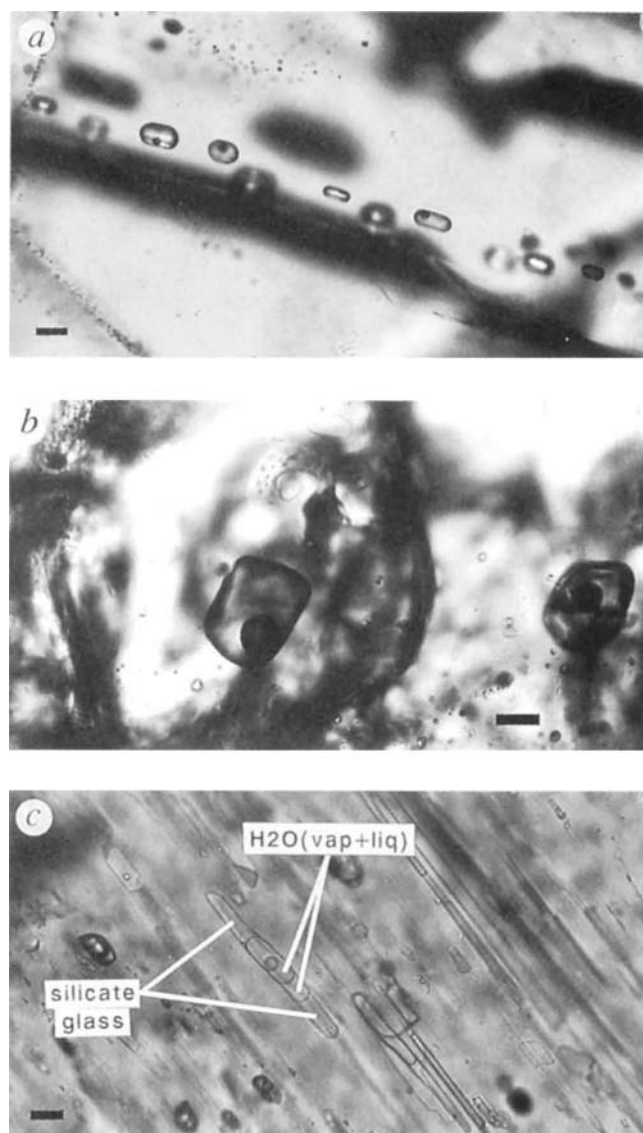


FIG. 2 Photomicrographs of high-silica glass inclusions in minerals from Batan peridotite nodules. a, Rounded-shape glass inclusions occur as trails along secondary fissures in olivine porphyroclasts. Their secondary nature implies that melt infiltration occurred in the residual depleted mantle wedge. b, Isolated inclusion with faceted shape occurs in an olivine neoblast from a hydrous-mineral-bearing peridotite. This primary inclusion corresponds to the parental liquid trapped during host mineral growth. c, Composite inclusions consisting of silicate glass + H_2O (vap + liq). Their occurrence suggests the former existence of an H_2O -rich silicate melt phase. Scale bar, $20\ \mu\text{m}$.

TABLE 1 Representative analyses of mineral phases in high-silica glass inclusions

	A	B	C	D	E	F	G
TiO ₂	1.85	0.30	0.03	0.45	1.43	0.29	0.22
Cr ₂ O ₃	1.27	0.05	0.33	0.41	0.39	44.98	0.11
MnO	0.17	0.06	0.31	0.23	0.08	0.30	0.10
FeO	6.49	9.24	9.52	3.78	6.98	22.63	10.65
K ₂ O	1.10	1.18		0.03	8.79		
CaO	11.97	10.01	0.71	22.51	0.08		0.05
Na ₂ O	1.85	1.56		0.22	0.85		
Al ₂ O ₃	12.87	12.90	1.23	3.20	16.72	16.41	65.04
P ₂ O ₅	0.01	0.18			0.01		
SiO ₂	42.20	48.96	55.49	51.72	39.94	0.06	0.03
MgO	16.60	13.56	32.21	16.19	21.26	12.61	22.80
Cl (p.p.m.)	<300	1140			<300		
Sum	96.49	98.02	99.82	98.73	96.52	97.28	99.00

A, B, amphibole (pargasite, hornblende); C, orthopyroxene; D, clinopyroxene; E, phlogopite; F, chromite; G, Al-rich spinel. Mineral compositions were measured with a CAMECA SX 50 electron microprobe (Camparis, Paris).

spinel harzburgites with porphyroclastic textures indicate an initial high-pressure plastic deformation episode which affected an already depleted mantle. (2) The occurrence of secondary trails of melt inclusions in olivine and orthopyroxene porphyroclasts implies that melt infiltrated into the depleted mantle rocks. (3) These melts precipitated the metasomatic minerals containing primary glass inclusions, which are portions of the parental liquids. The presence of primary H₂O-fluid and sulphide inclusions in metasomatic minerals and the systematic occurrence of solid sulphur in H₂O fluid inclusions indicates that there was exsolution of a sulphur and H₂O-rich fluid phase during crystallization. This exsolved fluid phase, which may correspond to that

previously identified⁴, probably induced melting of the metasomatized peridotites. (4) Finally, the metasomatized peridotites (including the metasomatic melts) were incorporated in the host basalt during ascent.

All xenoliths show widespread evidence of late, small-scale partial melting, generally occurring along orthopyroxene and spinel grain boundaries. The resulting melt patches have been partly recrystallized into small grains of olivine and clinopyroxene in a quenched glass. Colourless interstitial glasses often contain empty vesicles which are considered to have been previously filled by fluid. This melting episode clearly postdates the introduction of the primary metasomatic melts described below.

The major-element compositions of quenched, homogenized and glassy inclusions trapped in olivine porphyroclasts and neoblasts were determined by electron microprobe (Table 2). They exhibit calc-alkaline characteristics, that is, normative quartz and hypersthene and low TiO₂ concentrations, and they typically contain high contents of SiO₂ (53.6–62.6%), Al₂O₃ (16.3–18.9%), alkalis (Na₂O, 3.2–5.1%; K₂O, 2.3–4.4%). These chemical variations reflect either binary mixing or a continuous evolution from high-silica to less silica-rich compositions, as illustrated by the straight correlations observed in binary diagrams (Fig. 3). The glass inclusions also have high levels of chlorine (1,620–7,330 p.p.m.) and sulphur (140–2,500 p.p.m.). Ion probe H₂O analysis (CRPG, Nancy) of three glass inclusions (80–100 µm wide) range between 4.4 and 5.2%. Heated and unheated glass inclusions have similar compositions, except for small FeO and MgO enrichments in the former. Differences in these elements indicate post-entrapment host olivine crystallization, less than 6% (by weight) based on olivine–melt equilibrium¹².

Trace-element data from glass inclusions were obtained using an ion microprobe at the Woods Hole Oceanographic Institution

TABLE 2 Representative analyses of glass inclusions from Batan hydrous mineral-bearing xenoliths

	IV1	IV2	IV3	IV4	IV7	IVC	IVD	IVE	IVB	IVA1	IVA2	IVC2	IV12	IV13	mes.A	mes.B
SiO ₂ (wt%)	59.69	57.82	60.69	58.61	59.58	60.94	61.51	57.77	56.68	60.73	59.53	57.06	61.89	62.56	59.82	58.97
TiO ₂	0.12	0.00	0.13	0.07	0.16	0.19	0.02	0.32	0.20	0.24	0.35	0.44	0.84	0.44	0.25	0.24
Al ₂ O ₃	18.47	17.28	17.33	17.20	17.88	17.34	16.97	18.77	16.52	17.80	18.48	18.55	17.24	18.17	19.74	18.66
MnO	0.11	0.14	0.01	0.06	0.10	0.02	0.11	0.14	0.14	0.12	0.07	0.11	0.02	0.03	0.18	0.15
FeO	2.71	2.72	2.04	2.37	2.27	2.15	2.54	3.57	2.79	2.69	2.59	2.96	1.35	1.63	3.19	4.36
MgO	1.11	1.05	0.60	0.52	0.60	0.57	0.63	1.03	1.22	0.52	1.10	0.58	1.70	0.96	1.29	1.11
CaO	4.10	4.20	3.01	3.78	2.52	2.55	3.01	5.70	4.35	4.27	5.01	6.09	2.47	2.65	5.15	5.69
Na ₂ O	5.07	3.77	4.13	4.21	4.63	4.23	5.03	3.87	3.20	3.95	3.76	4.12	3.60	3.28	4.38	4.33
K ₂ O	2.47	2.61	3.14	2.92	3.14	3.60	2.46	3.75	3.04	3.20	2.67	2.28	4.04	4.76	3.12	3.23
P ₂ O ₅	0.36	0.34	0.34	0.51	0.23	0.32	0.38	0.70	0.16	0.38	0.37	0.71	0.02	0.28	0.73	0.59
Cr ₂ O ₃	ND	ND	ND	ND	ND	ND	ND	ND	0.11	ND	ND	ND	0.02	ND	ND	0.01
Cl (p.p.m.)	6860	1980	5420	5150	5670	2960	7330	4830	1850	3080	2950	4950	3460	2790	3280	4550
S (p.p.m.)	140	810	1060	530	940	630	700	750	1480	725	750	850	790	1350	20	30
SUM	94.91	90.62	92.06	90.83	91.78	91.95	92.74	96.18	88.96	94.27	94.28	93.47	93.41	95.30	98.17	97.81
HOST	Fe81	Fe81	Fe82	Fe81	Fe82	Fe83	Fe84	Fe81	Fe86	Fe81	Fe80	Fe80	Fe90	Fe92		
H ₂ O (wt%)	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.39	5.15	5.07	ND	ND	ND	ND
La (p.p.m.)	69.2	51.3	52.9	61.4	48.3	31.2	59.5	47.0	35.4						47.0	44.6
Ce	106.0	81.4	77.8	80.4	72.7	61.5	84.9	90.9	72.5						94.5	83.9
Nd	19.8	21.4	15.7	17.7	12.3	16.7	15.0	24.9	24.2						32.8	29.9
Sm	2.12	1.89	1.78	2.47	1.04	2.02	1.66	3.33	6.25						4.16	4.13
Dy	1.18	0.96	0.44	1.04	0.48	0.56	0.62	1.40	2.87						1.99	2.72
Yb	0.91	0.89	0.53	0.69	0.33	0.33	0.42	0.77	1.33						1.08	1.86
Ti	411	1576	910	1110	783	1509	618	1923	1424						2072	1809
V	53.8	71.6	27.6	70.5	37.8	51.0	54.5	127.8	91.3						106.2	85.1
Cr	14.6	74.5	16.3	5.5	4.5	40.2	44.6	58.8	70.6						25.1	6.9
Sr	713	279	519	810	637	355	762	477	412						450	501
Y	6.6	6.4	4.3	6.2	2.6	2.2	3.2	11.0	10.8						11.0	12.1
Zr	278.5	66.2	160	246.1	201.8	189.9	332.1	123	72.2						122.1	136.7
Nb	7.3	5.7	8.1	9.7	10.5	10.5	5.7	8.9	4.9						8.1	8.1

Analyses are of primary glass inclusions in olivine neoblasts (IV1–IVC2), secondary glass inclusions in olivine porphyroclasts (IV12 and IV13) and interstitial glasses (mes. A and mes. B). Glass compositions were measured with a CAMECA SX 50 electron microprobe (Camparis, Paris), using an accelerating voltage of 15 kV, sample current of 10 nA and a beam of 20–30 µm. To minimize volatilization of alkalis, Na was measured first during 10 s and the other elements were subsequently measured with counting times up to 60 s. Precisions of the Cl, S and Na analyses was established by replicate analyses of glass standards. Data for rare-earth elements (La, Ce, Nd, Sm, Dy, Yb), Zr, Y, Sr, Cr, V, Nb and Ti were obtained using a CAMECA IMS 3f ion microprobe (Woods Hole Oceanographic Institution) following techniques reported previously³⁵. H₂O was measured with a CAMECA IMS 3f ion microprobe (CRPG, Nancy). A negative primary oxygen beam of 0.5–2.5 nA intensity and maximum diameter of 20 µm, and energy filtering of -80 ± 10 eV were used. The counting rate of ¹H was standardized with respect to ³⁰Si peak, and a calibration curve was established between H₂O (wt%)/SiO₂ (wt%) and ¹H/³⁰Si ratios on standard glasses containing between 0.19 and 5.03 wt% H₂O; ND, not determined. Each average glass analysis for major elements represents the mean of 3–5 spot analyses. Error is <5%.

(Table 2; Fig. 1a). The glass inclusions have high concentrations of light-rare-earth elements (LREE), but concentrations of heavy-rare-earth elements (HREE) and Ti that are lower than average mid-ocean-ridge basalts (MORB)¹³. In addition, they have characteristic depletions of Nb and Ti relative to similarly incompatible elements, and their Ti/Zr ratios (1.5–19) are much lower than ratios from chondrites (115; ref. 14) or basalts from any tectonic environment (60–120; refs 2, 14).

Inclusion-bearing olivines have been analysed for trace elements by spark source mass spectrometry¹⁵ at the Max-Planck-Institut für Chemie in Mainz. In addition to the LREE enrichment and Nb depletion, the olivine separates have high contents of very incompatible elements (Cs, Rb, Ba, Th and U) and display strong positive anomalies of Pb, Sn and Cu (Fig. 1b). Ce/Pb ratios (0.7–2.2) are lower than typical Ce/Pb ratios (25 ± 5) for mid-ocean-ridge and ocean island basalts¹⁶ but are similar to the values obtained for arc lavas (1–10; ref. 17).

The compositions of the glass inclusions differ significantly from those of the Batan andesites. As well as having higher SiO₂ and alkali levels, the inclusions are more depleted in HREE, their negative HFSE anomalies are larger, and their Ti/Zr ratios are lower than those of the host lavas. The 'daughter' minerals

and the chemical composition of the silicate inclusions clearly indicate that they relate to the input of small amounts of a metasomatic melt phase, which altered the bulk chemistry and the mineralogy of the host peridotites.

Metasomatic melts migrating through the mantle wedge beneath Batan and those migrating through the oceanic and continental intraplate upper mantle^{18–22} are both characterized by high levels of silica. However, differences between the two types of melts allow us to distinguish metasomatic agents in subduction settings from those in within-plate settings. Metasomatic melts trapped as glass inclusions in intraplate continental and oceanic xenoliths are characterized by high levels of CO₂ (refs 18, 22). This is indicated by CO₂ oversaturation of the melt and cogenetic relationships with CO₂-fluid inclusions and carbonate inclusions. These features indicate the existence of a homogeneous carbonate-silicate melt, which later unmixed into two separate melts²². In contrast, metasomatic melts trapped in the Batan arc xenoliths show evidence for a (H₂O + S)-rich initial composition. Moreover, intraplate metasomatic melts crystallize Ti-rich kaersutite, diopside, rutile, ilmenite and magnesite¹⁸, a paragenesis clearly different from that produced by arc metasomatic melts. Finally, homogenization temperatures obtained for

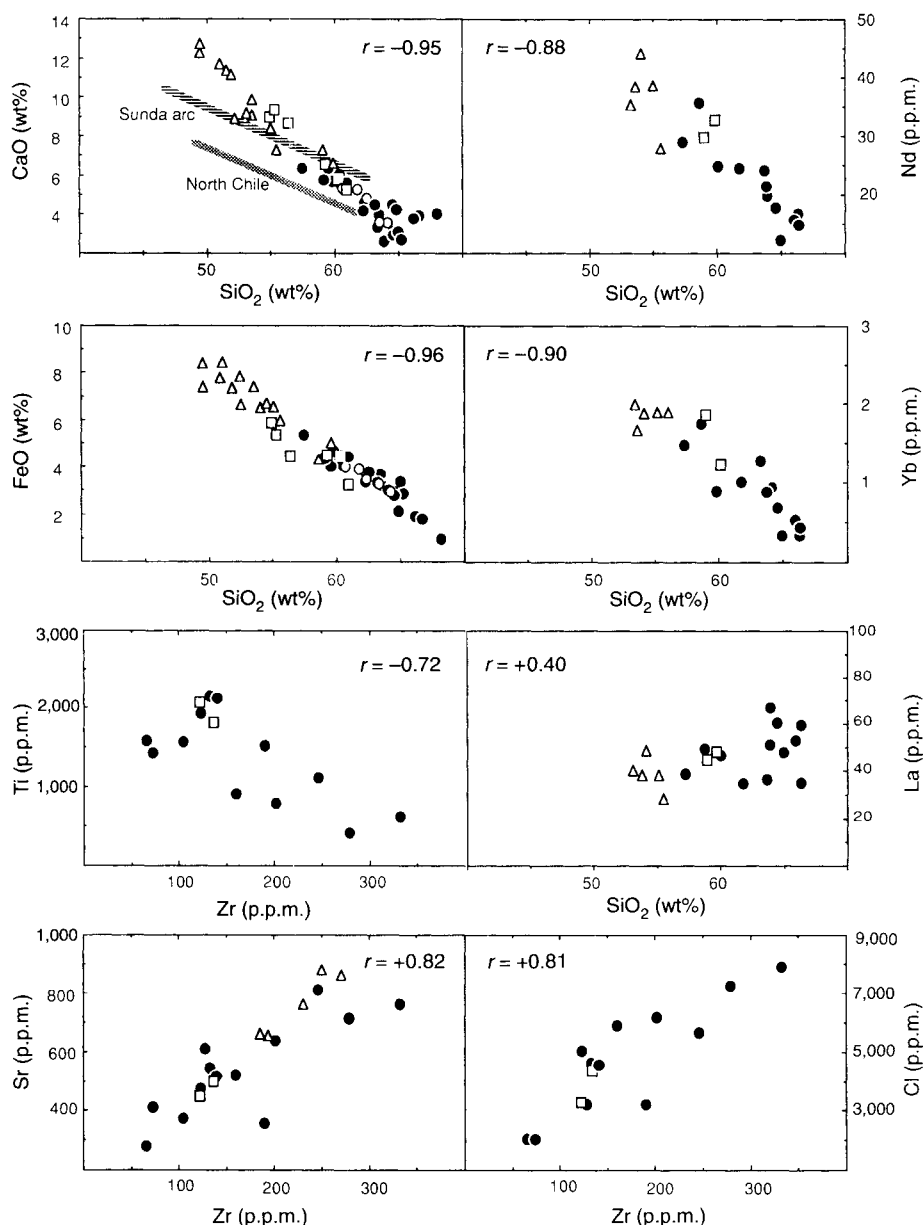


FIG. 3 Representative major and trace-element variation diagrams for unheated glass inclusions (filled circles) and heated inclusions (open circles) trapped in olivine porphyroclasts and neoblasts, interstitial glasses in metasomatized xenoliths (open squares) and Mount Iraya host basaltic andesites^{10,23} (open triangles). Unrelated arc magmas (Nevados de Payachata, North Chile³⁶ and Sunda arc, Indonesia³⁷) display trends significantly different from those defined by Batan andesites in the SiO₂ versus CaO figure. This indicates that the compositions of the Batan lavas are unique end-members of the linear trends. Major-element concentrations have been recalculated on an anhydrous basis; *r*, correlation coefficient.

melt inclusions in the two different settings (1250 °C for intra-plate melts and 920 °C for arc melts) also indicate differences in the genesis of these two metasomatic melts.

High-silica melt/basalt relationships

The glass inclusions trapped in olivine crystals and Batan calc-alkaline lavas (restricted to Mount Iraya host lavas^{10,23}) show linear chemical trends on variation diagrams (Fig. 3), suggesting that they have a common origin. This relationship is manifested by good correlations between major elements (recalculated on an anhydrous basis) and trace and volatile elements.

The linear trends shown in Fig. 3 might, at first sight, be interpreted to be the result of progressive fractional crystallization of an initially mafic liquid. However, this hypothesis is not consistent with the inverse correlations between SiO₂ and incompatible elements such as the REE, Ti or Zr. When a mafic magma is fractionated by progressive crystallization dominated by olivine, incompatible elements such as Nd or Zr should be progressively enriched, not depleted, as the residual melt fraction decreases.

The observed correlations are consistent with the hypothesis that the initial, lowest melt fraction is highly silicic, and that the concentrations of incompatible elements actually increase with progressively increasing melt fractions and progressively lower silica contents. This apparently paradoxical result is obtained if the bulk partition coefficients for the incompatible elements decrease with decreasing silica content of the melt²⁴, and if the magnitude of the melt fractions is lower than that of the bulk partition coefficients. In the extreme case, where $F \ll D$, the equilibrium-relation²⁵ $C_l/C_0 = 1/[D + F(1-D)]$ reduces to $C_l/C_0 \approx 1/D$, where C_l and C_0 are the concentrations in the liquid and in the bulk system, D is the bulk partition coefficient, and F is the melt fraction. However, if $F \gg D$, then $C_l/C_0 \approx 1/F$. Thus, for a given range of F , the resulting negative correlations with silica should be more pronounced for elements with relatively high values of D , such as Yb, than for elements with very low D , such as La, and the observed covariations are qualitatively consistent with this expectation (Fig. 3). Alternatively, the correlations seen in Fig. 3 are also consistent with a two-component mixing relationship, where one end-member is the host magma and the other is the silica-rich glass inclusions. Finally, combinations of these two mechanisms are also feasible and consistent with the data. In any case, the correlations appear to establish a common origin for the host andesites and the glass inclusions. Our conclusion that the high-silica melts are primary liquids that form an end-member of a related series of melts receives additional support from recent melting experiments on peridotites, where high concentrations of SiO₂ and Na₂O have been observed at very low melt fractions²⁶.

The metasomatic effect of the infiltrating melts on the major-element composition of the xenoliths is illustrated in Fig. 4, which shows a positive correlation between silica content of the melts and the forsterite (Mg/(Mg + Fe)) value of the host olivine. Evidently, the amounts of melts corresponding to the lowest melt fractions and having the highest silica contents were insufficient to change the initial olivine composition significantly (given recent experimental data²⁶, it seems possible that these extremely small melt fractions have reached equilibrium with their host olivine). Larger amounts of melts representing higher melt fractions and having lower silica and higher FeO contents then progressively reduced the forsterite content of the host olivine, producing metasomatized peridotites.

The metasomatic process, accompanied by small-scale melting of the xenoliths, is also recorded by interstitial glasses found in the xenoliths. Interstitial glasses have high SiO₂ and LREE concentrations ($(\text{La}/\text{Yb})_N = 17\text{--}30$), and depletions of Nb and Ti (Table 2; Fig. 1c). Their trace-element compositions, which differ from those of the glass inclusions ($(\text{La}/\text{Yb})_N = 40\text{--}105$), are close to the Batan calc-alkaline lavas ($(\text{La}/\text{Yb})_N = 10\text{--}20$). Moreover, the interstitial glasses fall on the trends defined by

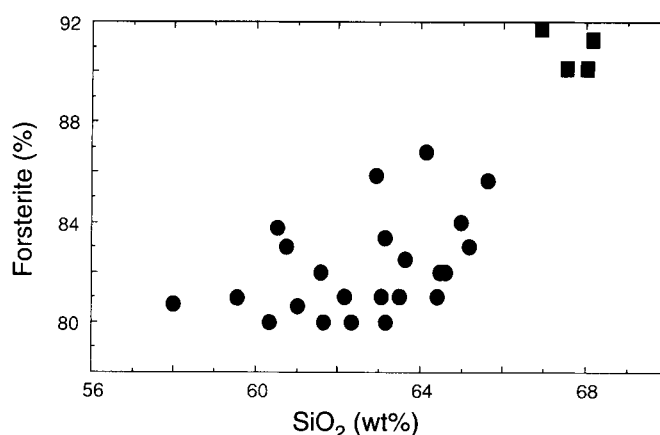


FIG. 4 SiO₂ content of the glass inclusions versus forsterite (Mg/(Mg + Fe)) value of the host olivine. Squares, secondary inclusions in olivine porphyroclasts; circles, primary inclusions in olivine neoblasts.

glass inclusions and Batan calc-alkaline lavas on variation diagrams (Fig. 3), suggesting that reaction between melts and peridotites leads to the development of intermediate liquids similar to the calc-alkaline lavas.

In summary, the existence of linear chemical trends between glass inclusions, interstitial glasses in xenoliths and the host lavas clearly indicates that all three melts have a common origin. This origin could, in principle, be one of simple progressive melting, but is more likely to be one of mixing between very low-degree slab-derived melts with high silica content, and higher degree melts generated in the mantle region previously metasomatized by the high-silica liquids. This common origin is further emphasized by the trace-element composition of the olivine separates (Fig. 1b), which is dominated by the glass inclusions and shows the negative Nb and positive Pb anomalies that are typical of most arc magmas. This indicates that partial melting of the metasomatized mantle source generates typical island-arc basalts rather than high-Nb basalts²⁷.

Slab-derived origin for the melts

The SiO₂-rich-end-member of the trends shown in Fig. 3 has silica-saturated dacitic composition, very low HREE (Yb < 0.35) and Y concentrations (Y < 3.0), and high LREE/HREE and Sr/Y ratios. Experimental results (see, for example, ref. 28) indicate that partial melting of subducted metabasalts may occur at the garnet amphibolite-eclogite transition at minimum pressures of 10 kbar (ref. 29) and temperatures of about 1,000 °C. This partial melting generates relatively high-aluminium, mostly corundum-normative silicic melts (Al₂O₃ > 15% at 70% SiO₂) with hornblende, clinopyroxene and garnet as residual minerals. The trace-element signature expected from these slab-derived magmas will be characterized by low HREE and Y concentrations, and high Sr/Y (> 40) and La/Yb (> 20) ratios^{2,30,31}. This is exactly what we observe in the Batan glass inclusions. Also, melting temperatures of metabasalts agree with the temperatures obtained during homogenization of glass inclusions (~920 °C). The flat, heavy REE patterns observed for glass inclusions could be related to the occurrence of amphibole as main residual phase³², and the high Zr abundances with low Ti/Zr values observed for glass inclusions could reflect the relatively high partition coefficient for Ti between amphibole and silica-rich melts³³. Finally, Batan peridotite nodules display MORB-type $\delta^{18}\text{O}$ values⁹.

Our observation of hydrous high-silica glass inclusions in the Batan nodules, showing the trace-element signature of slab-derived melts, lends direct support to the model of slab-mantle interactions, first proposed by Green and Ringwood¹, and Nicholls and Ringwood³⁴. The basaltic crust of the subducted

lithosphere undergoes a small degree of partial melting and dehydration. The resulting hydrous siliceous melts percolate through the overlying depleted mantle wedge. They thus metasomatize and 'refertilize' the depleted peridotites, and impart on them the trace-element source characteristics of typical island-arc magmas. This fertilized peridotitic region then melts to produce arc basalts and andesites. □

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LETTERS TO NATURE

The observational case for a low-density Universe with a non-zero cosmological constant

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OBSERVATIONS are providing progressively tighter constraints on cosmological models advanced to explain the formation of large-scale structure in the Universe. These include recent determinations of the Hubble constant^{1–3} (which quantifies the present expansion rate of the Universe) and measurements of the anisotropy of the cosmic microwave background^{4,5}. Although the limits imposed by these diverse observations have occasionally led to suggestions⁶ that cosmology is facing a crisis, we show here that there remains a wide range of cosmological models in good concordance with these constraints. The combined observations point to models in which the matter density of the Universe falls well below the critical energy density required to halt its expansion. But they also permit a substantial contribution to the energy density from the vacuum itself (a positive 'cosmological constant'), sufficient to recover the critical density favoured by the simplest inflationary models. The observations do not yet rule out the possibility that we live in an ever-expanding 'open' Universe, but a Universe having the critical energy density and a large cosmological constant appears to be favoured.

Cosmological models can be categorized according to their mechanism for generating seeds for the formation of large-scale structure. The standard Big Bang model successfully explains the Hubble expansion, the primordial formation of the elements, and the origin of cosmic background radiation. However, it offers no explanation for how structure formed. Recognizing that the Big Bang picture is incomplete, cosmologists have put forth various theoretical proposals to address that issue.

Our focus will be on a leading candidate, the inflationary model of the Universe^{7–9} although our analysis also extends to current alternatives. The inflationary model proposes that the seeds for large-scale structure formation were produced by microscopic quantum fluctuations in the energy density during the first instants after the Big Bang^{10–13}. There was subsequently a burst of spectacular, superluminal expansion (inflation) that stretched the Universe and the fluctuations to cosmic dimensions. The resulting spectrum of primordial fluctuations is nearly scale-invariant: if the fluctuation in density over space is expressed as a Fourier sum of waves with amplitude $\delta(\lambda)$, the waves have nearly equal amplitude independent of wavelength, λ . Cosmologists parametrize the spectrum in terms of a spectral index n , defined by the relation $\delta \propto \lambda^{(1-n)/2}$. In the early 1970s, before the inflationary model was proposed, Harrison¹⁴, Zel'dovich¹⁵ and Peebles and Yu¹⁶ had argued that a scale-invariant spectrum ($n=1$) is the most plausible because the amplitude did not diverge at small wavelengths, which would produce too many black holes, or at large wavelengths, which would produce too much distortion in the cosmic background radiation. Hence, it was regarded as a major triumph when it was discovered that inflation naturally generates a nearly scale-invariant spectrum.

It should be emphasized, however, that inflation does not predict a precisely $n=1$ spectrum. Rather, depending on the rate of inflation and the details of how inflation ends, the spectral index can take values between roughly $n=0.7$ and 1.2 (refs 5, 17). It is an important aspect of our tests that we do not fix the spectral index *ab initio*, but rather treat it as a free parameter to be constrained by observational data. In particular, we have found cases in which models have been judged inconsistent with large-scale observations under the strict assumption that $n=1$ (ref. 18). Yet a relatively modest deviation of n from unity, well within the bounds permitted by inflation, brings the model back into concordance.

Models can be further distinguished by the values of other cosmological parameters such as the Hubble expansion rate, H_0 , the density of baryons (ordinary matter), the total matter density including any dark matter, and the vacuum energy density or, equivalently, the cosmological constant (Λ). The symbols Ω_B ,

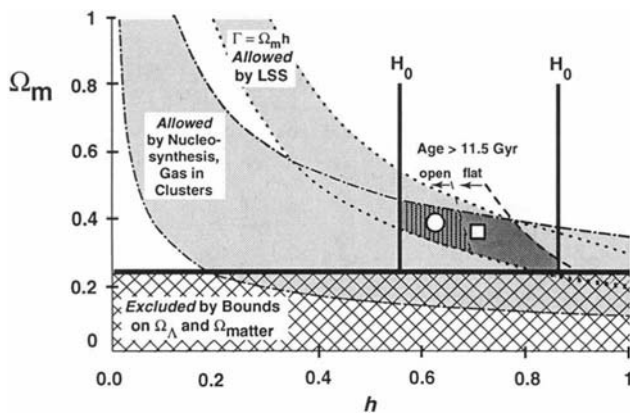


FIG. 1. The range of models in concordance with the best known astronomical observations. The entire darkly shaded region (with and without vertical stripes) is the concordance domain for flat models with $\Omega_m + \Omega_\Lambda = 1$; the vertically striped subregion alone applies to open models with $\Omega_\Lambda = 0$. (The age constraint—which permits the region only to the left of the dashed line—differs in the two cases.) The square indicates a central, representative flat model with $h = 0.7$, $\Omega_m = 0.35$ and $\Omega_\Lambda = 0.65$. The circle represents a representative open model, $h = 0.625$, $\Omega_m = 0.375$. See text for discussion of the constraints.

Ω_m , and Ω_Λ , are used to represent the ratio of baryons, total matter, or vacuum energy density to the critical energy density. The baryon density is constrained to $\Omega_B < 10\%$ by fitting Big Bang nucleosynthesis to the observed nuclear abundances¹⁹. An important property of inflationary expansion is that it produces sufficient energy to drive the Universe towards the critical energy density, $\Omega_{\text{total}} = \Omega_m + \Omega_\Lambda = 1$. Models with critical energy density are termed 'flat' because Einstein's theory of gravity predicts that these universes possess no overall curvature. Within this framework, conventional models have typically assumed that $\Omega_\Lambda = 0$ and $\Omega_{\text{total}} = \Omega_m = 1$, where the matter density consists of normal, baryonic matter plus cold (non-relativistic) dark matter, hot dark matter (relativistic at the onset of galaxy formation), or mixtures of the two²⁰. A second possibility is that $\Omega_{\text{total}} = 1$, with a non-zero cosmological constant, $\Omega_\Lambda > 0$, and Ω_m significantly less than unity. Yet a third possibility is that Ω_{total} is less than unity and the Universe is open. Although inflation is an extraordinarily efficient mechanism for driving Ω_{total} towards unity, some have considered 'open inflation' models^{21,22} in which the expansion is delicately adjusted to bring the Universe just short of critical density.

Our approach has been to apply objectively the best known observational constraints to the leading models and map out the permitted range of parameters. We first consider key astronomical measurements that delimit the plane of Ω_m versus H_0 (Fig. 1).

Measurements of H_0 . Most recent observations²³ are in the range $H_0 = 70 \pm 15$ kilometres per second per megaparsec ($\text{km s}^{-1} \text{Mpc}^{-1}$), the weighted average of the recent Hubble Space Telescope study using classical Cepheid variables¹ ($H_0 = 82 \pm 17 \text{ km s}^{-1} \text{Mpc}^{-1}$) and studies using type I supernovae² ($H_0 = 67 \pm 7 \text{ km s}^{-1} \text{Mpc}^{-1}$) as standard candles to infer distance.

Age of the Universe. The lower bound on the age of the Universe is based on re-evaluations of the ages of the oldest globular clusters: $t_0 = 15.8 \pm 2.1$ billion years based on the main-sequence turnoff⁶ and $t_0 = 13.5 \pm 2.0$ billion years using giant-branch fitting²⁴. The first limit, which suggests $t_0 > 13.7$ billion years at the 1σ level, is more stringent and, as it virtually excludes open models, is stronger support for our conclusions. However, to be conservative, we adopt the latter lower bound, 11.5 billion years, in Fig. 1.

Ω_Λ and Ω_m . Ω_Λ is constrained by many tests²⁵, but most directly by the measures of the number of gravitationally lensed quasars

versus redshift^{26,27}. For increasing Ω_Λ , the physical volume associated with an interval of redshift is larger, and hence the probability of encountering a lensing galaxy is greater. The bound $\Omega_\Lambda < 0.75$ is also consistent with the lower bound $0.2-0.3 < \Omega_m$ based on observed light density and cluster mass-to-light ratios²⁸ or by utilization of large-scale structure measurements²⁹. The two limits are represented as a cross-hatched region in Fig. 1.

Gas in clusters and nucleosynthesis. Assuming that galaxy clusters contain a representative baryonic and matter density, X-ray measurements of gas combined with estimates of the total virial masses³⁰ place a limit on the ratio of baryonic matter to total mass, $\Omega_B h^{3/2} / \Omega_m = 0.07 \pm 0.03$, where $H_0 \equiv 100h \text{ km s}^{-1} \text{Mpc}^{-1}$. Light-element nucleosynthesis¹⁹ constrains $\Omega_B h^2 = 0.015 \pm 0.005$. Together, these imply $1 - \Omega_\Lambda = \Omega_m = (0.21 \pm 0.12)h^{-1/2}$.

$\Gamma \equiv \Omega_m h$. The formation of large-scale structure depends on the primordial spectrum of inhomogeneities and also on cosmological parameters that determine how those seeds evolve in time. Fits assuming Ω_m consists of cold dark matter³¹ to the observed spectrum of mass fluctuations constrain the combination, $\Gamma \equiv \Omega_m h = 0.25 \pm 0.05$.

The range of concordance consistent with these constraints is indicated in Fig. 1. The heavily shaded region, both with and without vertical stripes, is for flat models with $\Omega_\Lambda > 0$. The vertical stripes indicate the somewhat more restricted subregion that applies for open models with $\Omega_\Lambda = 0$, with the reduction being due to a different age constraint. Two conclusions are worth noting here: (1) a substantial permitted area does exist, and (2) removal of any one of the observational sets of limits does not significantly enlarge the permitted area. Or, put differently, recovering $\Omega_m = 1$, as assumed in the standard cold, hot, and mixed dark-matter models, requires a combination of several observations to change in a coherent fashion. Independent analyses using some of the same astronomical tests have reached similar conclusions³²⁻³⁷.

A great leap forward in testing cosmological models is now possible due to the recently acquired ability to measure the cosmic background radiation (CBR) anisotropy. Because the CBR emanates from earlier epochs and greater distances than are probed by astronomical tests, the anisotropy provides truly independent information capable of discriminating among the surviving possibilities.

The amplitude of the CBR power spectrum, as determined by the Cosmic Background Explorer (COBE) satellite⁴, can be used to determine the spectrum of primordial fluctuations that seeded large-scale structure. The spectrum is specified by the spectral index n , which fixes the spectral shape, and an overall normalization, conventionally chosen to be σ_8 , the root-mean-square mass fluctuations averaged over eight $h^{-1} \text{Mpc}$ spheres. The value of σ_8 required to explain the distribution of large-scale structure and velocities³⁸ over 1–100 Mpc scales is $\sigma_8 = (0.56 \pm 0.06)(\Omega_m)^{-0.56}$. The COBE-determined amplitude is another measure of normalization, but based on the greater distances probed by the CBR. The extrapolation from COBE to a normalization at shorter distance scales depends on n , the fractional contribution of energy density fluctuations to CBR fluctuations, and the values of Ω_m , Ω_Λ , H_0 , and Ω_B (ref. 39). The latter parameters determine how fluctuations on $8h^{-1} \text{Mpc}$ scales evolve compared to COBE scales. Any given point in the concordance region of Fig. 1 corresponds to fixed Ω_m , Ω_Λ and H_0 , and Big Bang nucleosynthesis then determines Ω_B , by the constraints given above. Because inflation sets a relation between n and the energy density fluctuation contribution^{5,17}, matching COBE normalization to the astronomical normalization fixes the remaining undetermined parameter, the spectral index n .

We find that $-0.15 < n < 1 < 0.2$ for the flat models with $\Omega_\Lambda > 0$ which lie in the concordance region. This lies totally within the range of n determined by COBE⁴⁰ from comparing anisotropy averaged over a range of angular scales. It is also totally within the range that is achievable in inflationary models, n between

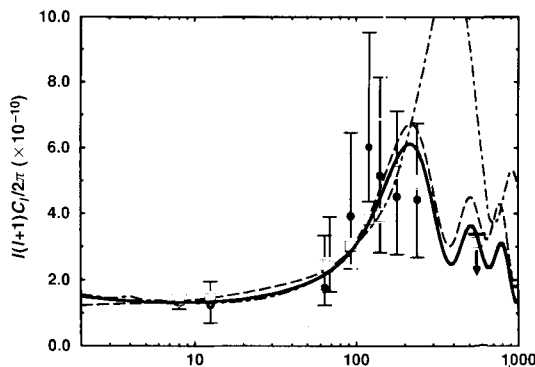


FIG. 2 Predicted CBR power spectrum for representative models: standard cold dark matter ($n=1$, $h=0.5$, $\Omega_\Lambda=0$; dashed line); a flat model in the concordance region of Fig. 1 ($n=0.96$, $h=0.65$, $\Omega_m=0.35$, $\Omega_\Lambda=0.65$, $\sigma_8=0.87$; solid line); and approximate results for an open model⁴³ in the concordance region, ($n=1.15$, $h=0.625$, $\Omega_m=0.375$; dot-dashed curve). The boxes represent the theoretical predictions for present CBR experiments for the flat model. The error bars represent 1σ detections, and the arrow indicates 95% upper confidence (no detection) limits. (See ref. 5 for details.) Note that the power spectrum for the flat model is remarkably similar to the prediction for standard cold dark matter, except at smaller angular scales ($l > 250$), where the flat model is marginally more consistent with present observational limits. Open models predict that the leftmost peak occurs at higher l and, consequently, has more power at small angular scales⁴³.

0.7 and 1.2 (refs 5, 17). For open models, the COBE fit⁴¹ requires shifting to n greater than unity, $+0.05 < n-1 < 1.4$. The larger value of n is required to compensate for the fact that there is less amplitude growth in open models compared to flat models. Requiring $n > 1$ is a new constraint on open models stemming from this analysis.

With Ω_m , H_0 and n determined for each type of model, CBR anisotropy measurements at intermediate scales (0.5–2°) can distinguish between the remaining models. The measurements determine the temperature autocorrelation function, $C(\theta)$, the sky-averaged product of the CBR temperature along two directions separated by angle θ . If $C(\theta)$ is re-expressed in Legendre polynomial series, $C(\theta) = (4\pi)^{-1} \sum (2\ell+1) C_\ell P_\ell(\cos \theta)$, the coefficients C_ℓ are called multipoles and a plot of $l(l+1)C_\ell$ is the CBR power spectrum. Roughly speaking, a given C_ℓ is determined by temperature variations across the sky with mean wavelength π/ℓ . The shape of the CBR power spectrum is very sensitive to models and their parameters. Figure 2 shows the CBR power spectrum for the standard cold dark matter model and for two representative models from the middle of the concordance regions, a flat model with $\Omega_\Lambda > 0$ (open square in Fig. 1) and an open model (open circle in Fig. 1)⁴². The important discriminating feature is the large Doppler peak, whose position along the abscissa is a sensitive test of $\Omega_m + \Omega_\Lambda$ and whose magnitude for the open concordance model is substantially greater than the Doppler peak for flat models²². The current results favour the flat models over the open models, although definitive conclusions

should be deferred until after the significant experimental improvements anticipated over the next few years.

Anisotropy measurements at yet smaller angular scales, down to 5-arcmin resolution or better, provide important corroborating evidence^{5,42}. An interesting feature, illustrated in Fig. 2, is that the predicted CBR power spectrum for $l > 250$ in standard ($\Omega_\Lambda=0$) cold dark matter models and in our flat concordance models are virtually indistinguishable and in equal agreement with observations, despite their substantially different cosmological parameter values. However, for $l > 250$ (spanning 10–30 arcmin), representative flat concordance models predict somewhat less power than standard cold dark matter models, marginally more consistent with current measurements by the OVRO, White Dish and MSAM experiments⁵. The open concordance models predict substantially more power than does the standard cold dark matter model⁵. This example is strong motivation for improved CBR experiments with angular resolution of 5–40 arcmin.

We would be interested to hear if a serious observational problem can be identified with the low-density concordance models illustrated in Figs 1 and 2 and, in particular, with the flat model which has a substantial cosmological constant. If not, perhaps we have already identified models which, in broad outline, capture the essential properties of the large-scale Universe. Should this be the case, a challenge arises: how can we explain the non-zero value of the cosmological constant from a theoretical point of view? □

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Detection of massive forming galaxies at redshifts $z > 1$

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THE complex problem of when and how galaxies formed has only recently become susceptible to direct attack. It has been known for some time that the excess of blue galaxies counted at faint magnitudes^{1–5} implies that a considerable fraction of the massive-star formation in the Universe occurred at redshift $z < 3$ (refs 6, 7); but surprisingly, spectroscopic studies^{8–11} of galaxies down to a B (blue) magnitude of 24 have found little sign of the expected high- z progenitors of current massive galaxies—finding instead mostly small blue galaxies at modest redshifts ($z \approx 0.3$). This unexpected population has diverted attention from the possibility that early massive galaxies in the process of star formation might also be found in the faint blue excess. From spectroscopic observations deep enough to encompass a large population of field galaxies (that is, those not associated with any cluster) at $z > 1$, we show here that in fact these forming galaxies are present in substantial numbers at $B \approx 24$, and that the era from redshifts 1 to 2 was clearly an important period of galaxy formation.

The recent availability of the highly sensitive multi-object Low Resolution Imaging Spectrograph (LRIS) on the Keck 10-m telescope on Mauna Kea, Hawaii has allowed us to extend and deepen quite considerably our spectroscopic coverage of the faint sky, and we are currently carrying out a redshift survey of faint galaxies using this instrument. The sample will consist of all objects satisfying the conditions $K < 20$ or $I < 22.5$ or $B < 24.5$ in four $6' \times 2'$ fields; so far, the most complete are those surrounding the Hawaii deep field SSA13^{4,12} in which 147 of the 174 objects have been observed and 124 of these securely identified, and around SSA22, with 186 of the 193 objects observed and 157 identified. In all, 91 galaxies lying beyond $z = 0.7$ and 40 beyond $z = 1$ have been identified in these two fields, with the highest redshift being $z = 1.69$. We show (Fig. 1) representative spectra of four galaxies at $z > 1$. Only three of the $z > 0.7$ sample

are galaxies with active nuclei (AGN). Most of the high-redshift identifications are based on the presence of a strong [O II] 3,727 Å emission line (a nebular emission line of singly ionized oxygen) and weak Mg II 2,800 Å absorption¹¹. The general form of the spectra is illustrated in more detail in Fig. 2, which is the average of all the $z > 0.7$ spectra in SSA13, excluding only the AGN. The composite spectrum closely resembles that of local blue galaxies^{13–15} with very strong [O II] 3,727, H β and [O III] 5,007, 4,959 Å emission lines in the optical, and ultraviolet absorption lines of Fe II, Mn II and Mg II that confirm the [O II] redshift identification. These absorption lines in the composite spectrum are redshifted by an average of 300 km s^{–1} from the emission lines, and may arise from gas falling into the galaxy.

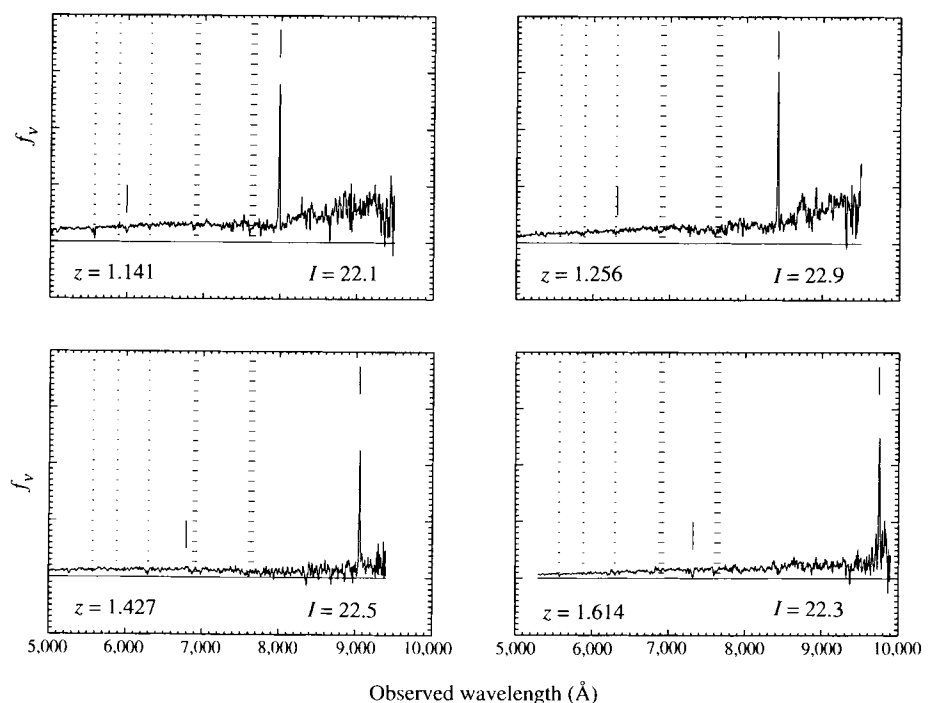
The [O II] 3,727 Å emission lines in the high-redshift objects are remarkably strong (rest equivalent width of 60 Å in the composite spectrum), given that these are luminous galaxies with absolute rest B magnitudes of about -21 for $H_0 = 50$ km s^{–1} and $q_0 = 0.5$. The [O II] luminosities ($L_{[\text{O II}]}$) of the individual galaxies in the two fields are shown (as squares and diamonds) in Fig. 3a, and we have also shown (as crosses) the [O II] luminosities for the Hawaii $K \leq 19$ sample¹⁶. At low redshift ($z < 0.7$) there are no galaxies in either sample with $L_{[\text{O II}]} > 10^{42}$ erg s^{–1} whereas at $z > 0.7$ there are 27 objects identified in these two fields. It is important to note that it is much easier to recognize and measure redshifts for objects with strong [O II], and that there may be $z > 1$ objects with lower [O II] luminosities hidden in the unidentified objects. There may also be additional objects at these redshifts lying beyond the magnitude limits of the present sample or among the remaining unobserved objects. This means the sample is not complete, but the important point is that even in this incomplete sample there is already a very large surface density of luminous star-forming galaxies.

To quantify this, we must translate the [O II] luminosities into galaxy formation rates. This is discussed by Gallagher *et al.*¹⁷ and by Kennicutt¹⁸, who point out that the [O II] line, although a more indirect calibrator than the unobserved H α , does provide a useful estimate of the star formation rate, expressed in solar masses ($M_\odot$) per year as:

$$\dot{M}(M_\odot \text{ yr}^{-1}) = 10^{-41} \alpha L_{[\text{O II}]}(\text{erg s}^{-1}) \quad (1)$$

We have introduced the parameter α to reflect the significant uncertainties in [O II]/H α ratios, internal extinction, and the

FIG. 1 Sample spectra of $z > 1$ galaxies observed with LRIS on the Keck 10-m telescope. Each object was observed with a 1.4-arcsec-wide slit using the 300 lines mm^{–1} grating, giving a resolution of 17 Å and a wavelength coverage of 5,000 Å. Exposure times range from one to four hours. Each object is shown as f_ν (continuum flux per unit frequency, with arbitrary normalization) versus observed wavelength, with short horizontal lines showing the positions of strong night-sky lines and atmospheric absorption bands. The vertical lines show the position of the Mg II 2,800 Å absorption and the [O II] 3,727 Å emission at the redshift shown in the lower left-hand corner of each panel. The apparent Kron-Cousins I magnitude is shown at the lower right-hand corner.



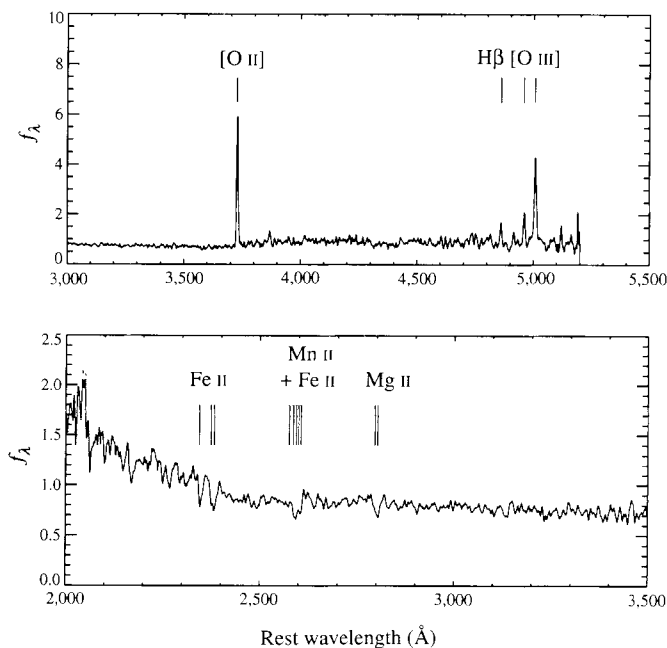


FIG. 2 The average rest-wavelength spectrum of objects with $z > 0.7$ in the SSA13 field (excluding AGN). This composite spectrum was formed by normalizing all the spectra to their median value, and then averaging all the spectra that covered a particular wavelength region. The optical portion of the spectrum (top panel) is characterized by strong emission lines, but only weak absorption lines are seen in the ultraviolet (bottom panel).

assumed stellar initial mass function. Gallagher *et al.* find $\alpha = 1$ based on a sample of nearby blue galaxies, whereas Kennicutt finds $\alpha = 5$ for a wider sample of types. This range of values is a good measure of the uncertainty, but the lower value of Gallagher *et al.* may be more representative of our present sample, which is extremely blue.

For $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and $q_0 = 0.5$, as assumed in Fig. 3, the age of the Universe at $z = 1$ is $6 \times 10^9 h^{-1} \text{ yr}$ ($h = H_0/$

$50 \text{ km s}^{-1} \text{ Mpc}^{-1}$) and formation of a 'normal' galaxy with $6 \times 10^{10} h^{-1} M_\odot$ of stars (a so-called L^* galaxy) would require a continuous star formation rate of $10 M_\odot \text{ yr}^{-1}$, corresponding to $L_{[\text{O II}]} = 10^{42} \text{ erg s}^{-1}$ for $\alpha = 1$ (the dashed line in Fig. 3a). We note again that such galaxies are not seen at $z < 0.7$ in the field samples (Fig. 2) or in either of the local^{17,18} samples, but are extremely common at $z > 0.7$. However, star formation need not proceed at a uniform rate in any individual galaxy, so the present observations might be picking out only the small subsample of starbursting galaxies at these redshifts. The best way to resolve this is to consider the volume production rate of [O II] photons by the ensemble of galaxies and compare this to the present mass density of stars in galaxies. This ensemble production rate of [O II] or H α photons directly measures the rate of massive star production and so can be linked immediately to the present-day density of metals^{6,7} in the Universe or, slightly more indirectly and subject to uncertainties in the stellar initial mass function, to the present-day mass density of stars in the Universe.

We first define the volume production rate of [O II] photons as

$$\mathcal{L}_{[\text{O II}]}(z) = \sum_{\Delta z} \frac{L_{[\text{O II}]}}{V_{\Delta z}} \quad (2)$$

where the sum is over all galaxies lying in the redshift interval Δz surrounding z , and $V_{\Delta z}$ is the co-moving volume corresponding to the observed area. *De facto* we are restricted in the summation to observed, identified galaxies and our measured values are lower bounds, with the correction being larger at high z . $\mathcal{L}_{[\text{O II}]}(z)$, which can be converted directly to a stellar mass density formation rate using equation (1), is shown in Fig. 3b for redshift intervals from $z = 0.25$ to 1.5. (It scales as h). We have distinguished entries for SSA13 (squares) and SSA22 (diamonds) to give some indication of the uncertainty, but evidently the total rate of (massive) star formation was much higher in the recent past: even without any further correction for missing objects, the star formation rate at $z = 1.125$ was four times higher than that at $z = 0.375$.

Turning to absolute values, we can compare the measured star formation rates with that required to form the presently observed star density. For reference purposes we assume that the current stellar mass density is $3 \times 10^8 h^2 M_\odot \text{ Mpc}^{-3}$ and that

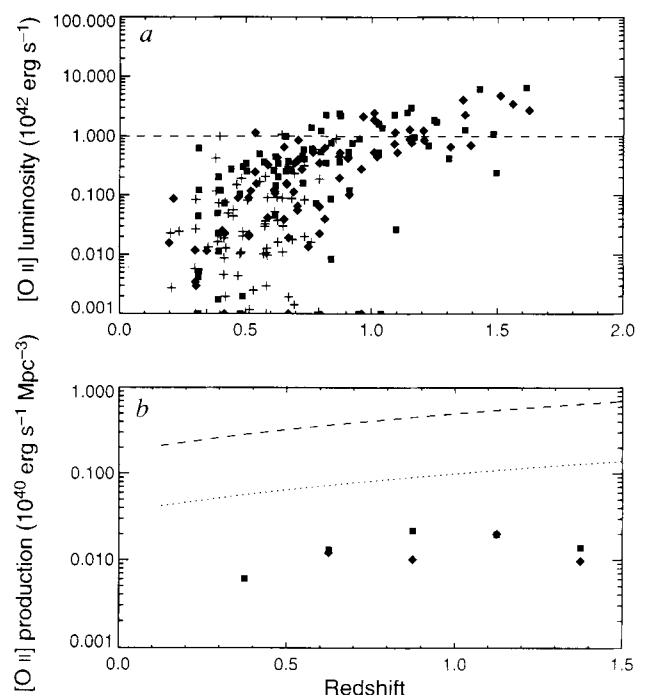


FIG. 3 *a*, The [O II] luminosities of galaxies in the SSA13 field (squares) and SSA22 field (diamonds), computed by normalizing each spectrum to the flux in the measured broadband magnitude nearest to the redshifted [O II] line to determine the continuum flux per unit wavelength, f_λ , and combining this with the luminosity distance at redshift z . The Songaila *et al.*¹⁶ sample are shown as crosses. The dashed line roughly divides rapidly forming galaxies from quiescent ones. No rapidly forming objects are seen at $z < 0.7$, but they become very common at higher redshift. *b*, The observed volume production rate of [O II] ($\mathcal{L}_{[\text{O II}]}$) as a function of redshift for SSA13 (squares) and SSA22 (diamonds) compared to the values required to form the present-day galaxy population. The lines show the calibration of star formation rate versus [O II] luminosity, as determined by Gallagher *et al.*¹⁷ (dashed line) and Kennicutt¹⁸ (dotted line). Some measure of the uncertainties can be obtained by comparing the two fields.

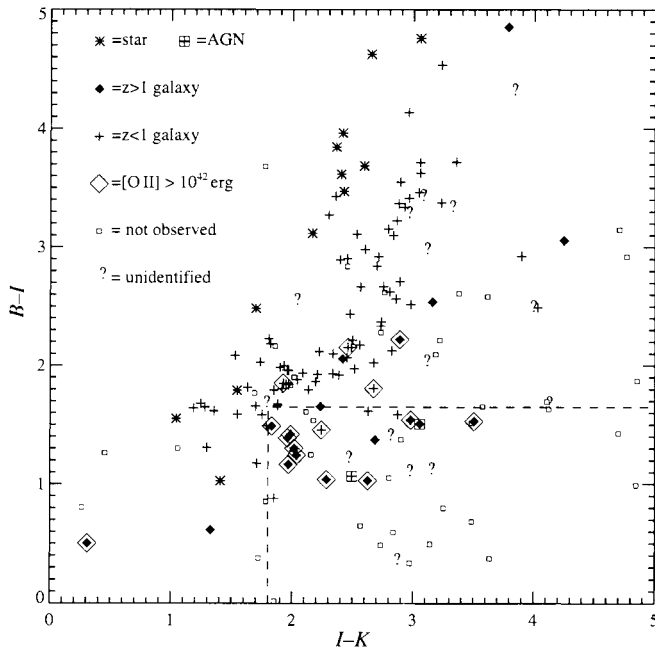


FIG. 4 All the 174 magnitude-selected objects in the SSA13 field, shown in the $(B-I)$ versus $(I-K)$ colour-colour plane. The dashed rectangle shows the portion of the colour-colour plane where most of the high- z $[\text{O II}]$ -luminous galaxies are found. Many of the unidentified, and most of the unobserved, galaxies also lie in this region of colour-colour space.

the available time is $17 h^{-1} (1+z)^{-3/2}$ Gyr. To form the current stars at some period then requires a star formation rate,

$$\dot{\mu}_{\text{ref}} = 1.7 \times 10^{-2} (1+z)^{3/2} h^3 M_{\odot} \text{Mpc}^{-3} \text{yr}^{-1} \quad (3)$$

This reference rate is shown as the dashed and dotted lines in Fig. 3b, which are computed for the values $\alpha=1$ and $\alpha=5$ respectively in equation (1). At $z>1$, even in this quite incomplete sample, we are already seeing between 4% and 20% of the total galaxy formation.

Most of the luminous $[\text{O II}]$ galaxies and most of the identified $z>1$ galaxies are drawn from objects with blue optical colours $[(B-I) \leq 1.7]$ but relatively red infrared colours¹² $[(I-K) \geq 1.8]$ (Fig. 4). Eleven of the 16 objects in SSA13 with $L_{[\text{O II}]} \geq 10^{42} \text{ erg s}^{-1}$ and 13 of the 20 objects with $z>1$ lie in this portion of the colour-colour plane; conversely, nearly all the identified objects in this region are predominantly $[\text{O II}]$ -luminous high- z galaxies. Of the 18 objects identified in these regions two are AGN and two are low- z galaxies, but all those remaining have $L_{[\text{O II}]} > 4 \times 10^{41} \text{ erg s}^{-1}$ and lie at $z>0.7$. Inspection of the colour-colour plane suggests that nearly all the low- z galaxies in the sample have been identified and the remaining unidentified or unobserved objects are luminous high- z star formers. Completion of the sample should therefore increase the $z>1$ galaxy formation rate substantially, bringing the measured mass rate much closer to the reference value.

We have used deep I -band images, obtained with the wide field camera (WFPC2) on the Hubble Space Telescope (HST), of the central regions of the SSA13 and SSA22 fields¹⁹ to investigate the morphologies of the high- z star-forming galaxies. The images of all nine known objects in the HST fields with $z>1$ and $L_{[\text{O II}]} \geq 10^{42} \text{ erg s}^{-1}$ are shown in Fig. 5. Unlike lower-redshift objects with high $[\text{O II}]$ equivalent widths²⁰, the objects have strikingly unusual morphologies, often consisting of chains¹⁹ or structures of compact blobs, suggesting that they are generally not dominated by uniformly distributed star formation. Recent studies have shown^{19,21} that the excess galaxy counts at faint

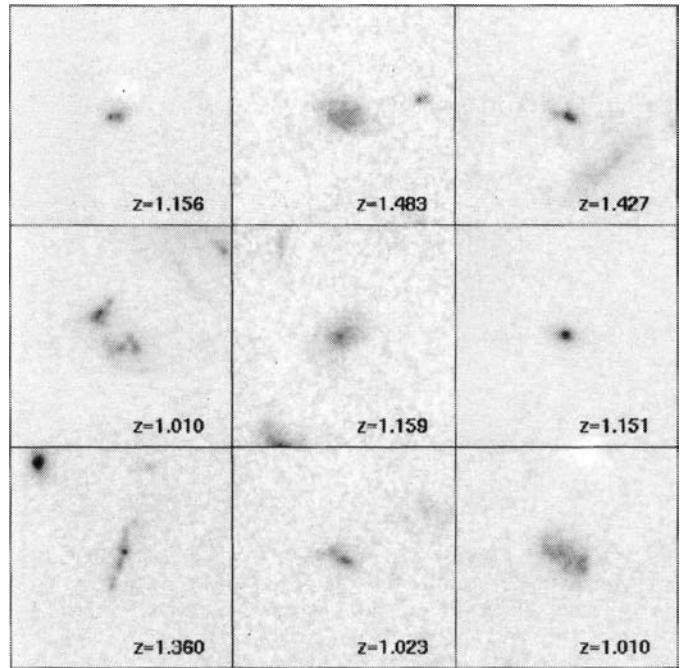


FIG. 5 Deep I -band images¹⁹ (obtained by the Hubble Space Telescope, HST) of all nine galaxies with $z>1$ and $L_{[\text{O II}]} \geq 10^{42} \text{ erg s}^{-1}$ that lie in the HST fields. The boxes are 8 arcsec on a side and the redshift of each object is shown in the lower right-hand corner of the panels.

blue magnitudes are dominated by these anomalous galaxies, and that at faint infrared magnitudes ($K>20$) these also become the single largest population¹⁹. Combined with the present results we can now recognize that forming galaxies enter the galaxy counts in substantial numbers at faint ($B>24$, $K>20$) magnitudes. $\square$

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Electron acceleration from the breaking of relativistic plasma waves

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ELECTRONS in a plasma undergo collective wave-like oscillations near the plasma frequency. These plasma waves can have a range of wavelengths and hence a range of phase velocities¹. Of particular note are relativistic plasma waves^{2,3}, for which the phase velocity approaches the speed of light; the longitudinal electric field associated with such waves can be extremely large, and can be used to accelerate electrons (either injected externally or supplied by the plasma) to high energies over very short distances²⁻⁴. The maximum electric field, and hence maximum acceleration rate, that can be obtained in this way is determined by the maximum amplitude of oscillation that can be supported by the plasma⁵⁻⁸. When this limit is reached, the plasma wave is said to 'break'. Here we report observations of relativistic plasma waves driven to breaking point by the Raman forward-scattering instability^{9,10} induced by short, high-intensity laser pulses. The onset of wave-breaking is indicated by a sudden increase in both the number and maximum energy (up to 44 MeV) of accelerated plasma electrons, as well as by the loss of coherence of laser light scattered from the plasma wave.

The maximum amplitude of a plasma oscillation can be obtained from nonlinear plasma theory. As the wave grows, its waveform is no longer sinusoidal, that is, it steepens. In the cold-plasma theory^{5,7}, wave-breaking occurs when this steepening is so extreme that there are singularities in the plasma density. In reality, thermal effects determine the wave-breaking amplitude^{6,8} for two reasons. First, the tendency of the plasma density to increase to infinity at the wave crests is opposed by plasma pressure, and second, at some large amplitude, plasma electrons with large thermal velocities along the direction of the phase velocity begin to be trapped and accelerated by the wave. A self-trapped electron is one that already has or picks up enough energy from the electric field of the wave to begin to move forward in the frame of the wave (to accelerate). Untrapped electrons simply move with their thermal velocity in addition to taking part in the coherent wave oscillations. At wave-breaking, the number of electrons being trapped is so large that these accelerating particles damp the wave irreversibly. As estimate of the maximum electric field for a relativistic plasma wave based on Gauss's law¹¹ gives $E = 0.96\alpha(n_e)^{1/2} \text{ V cm}^{-1}$ where n_e is the plasma density in cm^{-3} , and $\alpha = \tilde{n}/n_e$ is the normalized density perturbation or wave amplitude and is ~ 1 at wave-breaking in our typical conditions, and $\tilde{n}$ is the magnitude of the perturbation of the electron density associated with the wave. Neglecting any beam loading effects, the maximum amount of energy^{3,11} a trapped electron can obtain in such a wave is 'dephasing limited' to roughly $2\alpha\gamma_\phi^2 mc^2$ where $\gamma_\phi = (1 - v_\phi^2/c^2)^{-1/2}$ is the relativistic Lorentz factor associated with the phase velocity v_ϕ of the wave and m is the electron mass. Dephasing occurs when the accelerated electrons outrun the wave, limiting the useful interaction length. Observation of a sudden increase in both the number of

self-trapped electrons accelerated by the plasma and maximum electron energy close to the dephasing-limited value is a strong indication that wave-breaking has occurred.

In the experiments described here, the relativistic plasma wave is excited by an intense ($> 5 \times 10^{18} \text{ W cm}^{-2}$), short-duration ($< 1 \text{ ps}$), $1.054\text{-}\mu\text{m}$ -wavelength laser pulse via the Raman forward-scattering (RFS) instability^{9,10}. This is the decay (induced by a noise-level plasma wave) of the strong electromagnetic pump wave (ω_o, k_o) into a plasma wave (ω_p, k_p) and two forward-propagating electromagnetic cascades at the Stokes ($\omega_o - n\omega_p$) and anti-Stokes ($\omega_o + n\omega_p$) frequencies. Here $\omega_p = (4\pi n_e e^2/m)^{1/2}$ is the plasma frequency, n is a positive integer, e is the electron charge, and ω and k are the angular frequency and the wavenumber respectively, of the indicated waves. The spatial and temporal interference of these sidebands with the laser produces an electromagnetic beat pattern propagating synchronously with the plasma wave. The electromagnetic beat exerts a force on the plasma electrons, reinforcing the original noise-level plasma wave which then scatters more sidebands, thus closing the feedback loop for the instability⁹.

The early experimental work on RFS was performed using long laser pulses (0.1–2 ns) of substantially lower intensity ($< 10^{15} \text{ W cm}^{-2}$)^{12–14}. In these experiments, the RFS instability was in the spatial-convective (long-pulse) regime. Although self-trapped electrons were observed out to 1.4 MeV in one of these experiments¹², the possibility of driving the plasma wave to its maximum wave-breaking level in all three of these experiments was precluded by the low laser intensities and the short interaction lengths. More recent experimental work on RFS involved the newer, short-pulse laser technology but still with relatively low intensity^{15,16}. Acceleration of injected electrons was reported by Nakajima *et al.*¹⁵ and correlation of accelerated electrons with an anti-Stokes sideband was reported by Coverdale *et al.*¹⁶. In neither case did plasma waves grow to wave-breaking as evidenced by the absence of copious electrons near the theoretical maximum energy. Finally, an experimental study of wavebreaking of non-relativistic plasma waves induced by Raman backscatter⁹ was recently reported¹⁷. The main diagnostic, scattered light from an external probe beam, indicated destruction of the wave's coherence and trapping and acceleration of back-ground electrons (up to $4 v_\phi$ or $\sim 1 \text{ keV}$) was inferred by computer simulations.

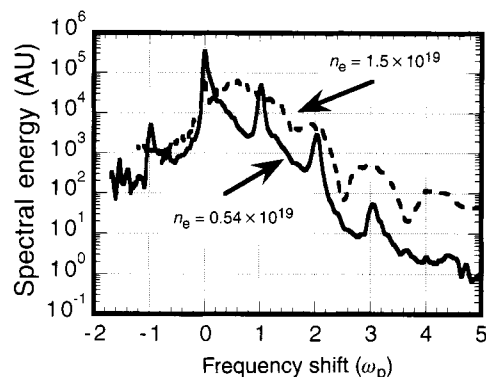


FIG. 1 Frequency spectrum of the light transmitted through the plasma in the $f/5$ cone angle of the focused laser beam for two different plasma densities: $0.54 \times 10^{19} \text{ cm}^{-3}$ (solid curve) and $1.5 \times 10^{19} \text{ cm}^{-3}$ (dashed curve); AU, arbitrary units. Because the frequency scale has been normalized to ω_p , the sidebands have integer values for both densities. The spectrum was read out with a 16-bit silicon CCD camera which has essentially no sensitivity at wavelengths $> 1.2 \mu\text{m}$. A bright blackbody source placed in the plasma location was used to measure the overall transmission function of the optics and the spectral response of the CCD camera, and this was used to correct the measured spectrum. The first Stokes sideband (at $\Delta\omega/\omega_p = -1$) in the low-density shot is just within the spectral sensitivity of the CCD camera, whereas the first Stokes sideband in the high-density shot is outside this sensitivity.

Other laser-driven plasma accelerators, namely the Plasma Wakefield Accelerator^{3,18} (PWFA) and the Plasma Beat Wave Accelerator^{3,4} (PBWA), have been discussed in the literature in the context of a future alternative technology to radio-frequency linear accelerators (linacs)¹⁹. In these schemes, wave-breaking is undesirable for issues relating to the beam quality of the output electron beam. Instead, a high-quality electron beam is injected into the plasma wave which is driven in a controlled manner by either a two-frequency laser beam (PBWA) or an extremely short (shorter than a plasma wavelength) laser pulse (PWFA) rather than through an instability of an intense, short (but longer than a plasma wavelength) laser pulse as is the case here. Recent experiments have reported an energy gain of about 13 MeV in the PWFA¹⁵ and gains in 10–30 MeV range in the PBWA^{20–23}.

When a sufficiently intense short-pulse laser drives the RFS instability, the instability is said to be in the spatio-temporal regime. In this case, the plasma wave grows substantially in the frame of the laser pulse as the pulse transits the plasma. This is in contrast to the long-pulse, low-intensity laser case where the plasma wave growth is small on the scale of a plasma-transit time (for typical plasma lengths). The spatio-temporal theory of RFS for short pulses predicts¹⁰ that the total gain G for the relativistic plasma wave growing from noise is given by $G = e^2 / (2\pi g)^{1/2}$ where $g = [a_0 / \sqrt{2(1 + a_0^2/2)}](\omega_p / \omega_0)^2 (\omega_0 / c) \sqrt{x} \psi$. Here, $a_0 = eA / mc^2$ is the normalized vector potential of the laser which is proportional to the square root of the laser intensity, x is the distance travelled into the plasma, and ψ / c is the length of time that the assumed constant-intensity pulse has interacted with the plasma at position, and A is the laser vector potential. To reach wave-breaking, that is $a \approx 1$, we must have $G a_n \approx 1$ where a_n is the initial noise level. Calculating this noise level for the general case is not possible because there are various complex mechanisms for generating the noise. For $n_e = 1.5 \times 10^{19} \text{ cm}^{-3}$ and $a_0 \approx 1$, the coupling between the laser and faster-growing (relative to RFS) but non-forward Raman scattering modes can be very strong and actually deplete a portion of the pump by scattering it into various angles^{16,24,25}. Simulations show that this pump depletion can be quite localized on the rising edge ($a_0 < 1$) of the pump and thus form a 'notch' on the pulse envelope after propagating only a fraction of Rayleigh length (the length over which a focused laser beam drops by a fraction of two in intensity) into the plasma^{24,25}. The plasma wave 'wake' associated with this notch moves with the laser and therefore will act as an enhanced noise level to seed the RFS instability^{24,25}. Because the instability growth rate g is weakly dependent on laser intensity for $1 < a_0 < 2$, we can take the interaction time $\psi / c \approx 800 \text{ fs}$ (the duration of the pulse for which $a_0 > 1$ at $x = 350 \mu\text{m}$). These parameters, along with a density of $n_e = 1.5 \times 10^{19} \text{ cm}^{-3}$, are sufficient to reach the wave-breaking condition $a_n G \approx 1$ by the end of the pulse if $a_n > 3 \times 10^{-5}$. Computer particle-simulations of this particular experiment²⁵ show that the noise level can be much greater than this value due to the above-described notching mechanism, leading to wave-breaking of RFS within a Rayleigh length for a smaller ϕ / c . Note that G is an extremely sensitive function of the electron density because g is proportional to n_e .

We report the results of an experiment where evidence for plasma wave-breaking has been observed using the VULCAN laser at Rutherford Appleton Laboratory²⁶ which provided 25-TW, 0.8-ps pulses on target with a 20- μm -diameter laser spot using $f/5$ optics. The effective Rayleigh length was 350 μm . The laser was focused onto the well-defined edge of a 4-mm-diameter, laminar plume of helium gas from a pulsed, supersonic gas jet²⁷ located 2 mm below the focal region. The maximum intensity of $6 \times 10^{18} \text{ W cm}^{-2}$ is sufficient to doubly-ionize the helium gas producing a fully-ionized plasma over at least 2 mm into the jet. The exponentiation rate $\ln(G(\phi, x))$ of the Raman instability, and therefore of the electron plasma wave, is approximately proportional to n_e . The plasma density was experimentally controlled by varying the backing pressure of the jet and was

measured (through the frequency shift of the anti-Stokes sidebands) to be linear with backing pressure from 5 bar to at least 18 bar where the electron density was $1.5 \times 10^{19} \text{ cm}^{-3}$. We expect that varying the density over this factor of ~ 3 should result in enormous changes in the final electron plasma wave amplitude. The two important diagnostics discussed here are the forward scattered electromagnetic spectra in the full $f/5$ for the incident laser cone and the energy spectra of trapped and accelerated electrons in an $f/60$ cone centred on the laser axis.

The solid curve in Fig. 1 shows the electromagnetic frequency spectrum emerging from the plasma with a density of $5.4 \times 10^{18} \text{ cm}^{-3}$ where the abscissa is the shift in frequency $\Delta\omega$ of the forward scattered light from the laser frequency in units of ω_p . The upshifted anti-Stokes and downshifted Stokes signals at $\Delta\omega / \omega_p = \pm 1$ are clearly visible as is the transmitted pump at $\Delta\omega / \omega_p = 0$ and the second and third anti-Stokes sidebands. These signals are sharply peaked, and their width indicates that the plasma wave which generated these signals must have a coherence time of the order of the laser pulse. The dashed curve in Fig. 1 shows the spectrum when the plasma density is increased to $1.5 \times 10^{19} \text{ cm}^{-3}$. The most startling feature is the tremendous broadening of the individual anti-Stokes peaks at this higher density. This broadening is thought to be characteristic of wave-breaking and is mainly caused by the loss of coherence due to severe amplitude and phase modulation as the wave breaks. As wave-breaking evolves, the laser light no longer scatters off a collective mode of the plasma but instead scatters

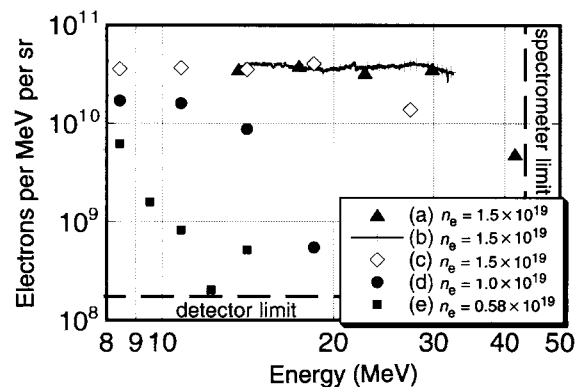


FIG. 2 The electron energy spectrum measured using a magnetic spectrometer in the forward $f/60$ cone centred on the laser axis for three different plasma densities: $1.5 \times 10^{19} \text{ cm}^{-3}$ (a–c), $1.0 \times 10^{19} \text{ cm}^{-3}$ (d) and $0.54 \times 10^{19} \text{ cm}^{-3}$ (e). All three cases were at a laser power of $\sim 25 \text{ TW}$. Curves a and b were taken on the same laser shot with the highest magnetic field available in the spectrometer, whereas curve c was taken on a separate shot at a lower field strength. The variance between the two shots between 27 and 30 MeV may be indicative of the shot-to-shot reproducibility of the data. All the data except for curve b were taken with biased silicon surface-barrier detectors. Curve b was obtained using a fluorescer/film combination, giving a continuous spectrum from 13 to 32 MeV. For this film data, the vertical scale was adjusted to overlay the solid triangles for the same shot. The absolute electron flux was estimated from the known energy deposition rate (one electron-hole pair per 3.6 eV) in the silicon detectors along with the known sensitivity (volts per coulomb) in the preamplifiers. The energy plane dispersion (cm MeV^{-1}) was used to convert the signal on the 0.7-cm-wide detectors (signal per cm) into signal per MeV and thus electrons per MeV. Finally, the points were normalized to the $f/60$ solid angle (electrons per MeV per sr) to facilitate comparison of these electron spectra to spectral data in the literature. A peculiar aspect of the measured spectrum at high density is that it is approximately flat from 15 to 30 MeV. This is due to the fact that the observation is made in only an $f/60$ cone in the exact forward direction. The highest energy electrons are those that traverse the wave in the region of the highest longitudinal fields, that is, right down the axis of the plasma wave. Lower-energy electrons necessarily experience more of the radial fields and would tend to have a larger angular spread as a result.

individually off the trapped electrons which are still periodically deployed in space (that is, still k -matched to forward scattering) but having a range of momenta producing, therefore, a range of scattered frequencies¹⁷. Also, the Doppler shifts²⁸ expected for electrons moving at, say, a relativistic $\gamma \approx 4$ within a range of angles corresponding to $f/5$ focusing is approximately ω_p and nearly symmetric on the red and blue sides of the satellites which could further explain the 'filling in' of the satellites.

Figure 2 shows the measured electron energy spectra corresponding to the two optical spectra in Fig. 1 plus an intermediate plasma density of $1.0 \times 10^{19} \text{ cm}^{-3}$. The number of accelerated electrons at a given energy and the maximum electron energy both show a dramatic increase as the plasma density is increased to $1.5 \times 10^{19} \text{ cm}^{-3}$: the number of electrons above 20 MeV increases by at least two orders of magnitude and the accelerated electron distribution is rather flat up to 30 MeV where it begins to decrease with energy up to 44 MeV, which is the spectrometer limit. We interpret this sudden increase in accelerated electrons and maximum energy, together with the broadening of the electromagnetic spectrum, as the signature that wave-breaking has occurred. The large increase in the number of electrons accelerated at the highest plasma density is consistent with the wave trapping the bulk of the plasma thermal distribution function rather than a few tail particles at low plasma densities. Indeed the (spectrometer-limited) maximum electron energy of 44 MeV is not too far from the absolute maximum of 70 MeV that a test electron would obtain, limited by dephasing in an ideal plasma wave with $\alpha = 1$ which is near the relativistic warm-plasma wave-breaking limit expected for our plasma conditions.

We note that the normalized transverse emittance $\varepsilon_n = \gamma \sigma \delta \theta$ of any particular energy group from this experiment is quite small. Here γ is the relativistic Lorentz factor for that energy group, σ is the source size ($\sim 10 \mu\text{m}$), and $\delta \theta$ the angular spread ($\sim 8 \text{ mrad}$ due to the $f/60$ collection). At 30 MeV, $\varepsilon_n = 5 \pi \text{ mm mrad}$ which is low enough to be competitive with modern photo-injector-based linacs²⁹. However, the beam current measured here ($\sim 1 \text{ A}$ in a $\pm 1\%$ bandwidth around 30 MeV) is roughly 10–100 times lower than present-day photoinjector technology. The tremendous advantage over conventional linacs is the extremely short distance over which this energy is obtained. As dephasing limits the acceleration length to $\pi \gamma^3 / k_0 \approx 300 \mu\text{m}$, the 44 MeV that the electrons gain indicate a peak electric field of over 100 GV m^{-1} which would represent the higher collective wave field ever produced in a laboratory. Given that laser technology, including repetition rate, average power, efficiency and smaller packaging is advancing very rapidly, it is reasonable to expect that the average current can be increased through a combination of higher laser frequencies and plasma densities as well as by increased repetition rate. Thus, one could envisage in the not-too-distant future a new class of compact accelerators based on the breaking of relativistic electron plasma waves which may find applications where 2–200 MeV electrons or photons are needed. $\square$

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Modelling urban growth patterns

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CITIES grow in a way that might be expected to resemble the growth of two-dimensional aggregates of particles, and this has led to recent attempts^{1–3} to model urban growth using ideas from the statistical physics of clusters. In particular, the model of diffusion-limited aggregation^{4,5} (DLA) has been invoked to rationalize the apparently fractal nature of urban morphologies¹. The DLA model predicts that there should exist only one large fractal cluster, which is almost perfectly screened from incoming 'development units' (representing, for example, people, capital or resources), so that almost all of the cluster growth takes place at the tips of the cluster's branches. Here we show that an alternative model, in which development units are correlated rather than being added to the cluster at random, is better able to reproduce the observed morphology of cities and the area distribution of sub-clusters ('towns') in an urban system, and can also describe urban growth dynamics. Our physical model, which corresponds to the correlated percolation model^{6–8} in the presence of a density gradient⁹, is motivated by the fact that in urban areas development attracts further development. The model offers the possibility of predicting the global properties (such as scaling behaviour) of urban morphologies.

In the model we now develop we take into account two points. First, data on the population density $\rho(r)$ of actual urban systems are known to conform to the relation¹⁰ $\rho(r) = \rho_0 e^{-\lambda r}$, where r is the radial distance from the compact core, and λ is the density gradient. Therefore, in our model the development units are positioned with an occupancy probability $p(r) \equiv \rho(r)/\rho_0$ that behaves in the same fashion as is seen in observations of real cities. Second, in actual urban systems, the development units are not positioned at random. Rather, there exist correlations arising from the fact that when a development unit is located in a given place, the probability of adjacent development units increases; each site is not independently occupied by a development unit, but is occupied with a probability that depends on the occupancy of the neighbourhood.

In order to quantify these ideas, we use the correlated percolation model^{6–8}. In the limit where correlations are so small as to be negligible^{11,12}, a site at position $\mathbf{r}$ is occupied if the occupancy variable $u(\mathbf{r})$ —an uncorrelated random number—is smaller than the occupation probability $p(\mathbf{r})$. To introduce correlations among the variables, we convolute the uncorrelated variables $u(\mathbf{r})$ with a suitable power-law kernel⁷, and define a new set of random variables $\eta(\mathbf{r})$ with long-range power-law correlations that decay as $r^{-\alpha}$, where $r \equiv |\mathbf{r}|$. The assumption of power-law interactions is motivated by the fact that the 'decision' for a

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development unit to be placed in a given location decays gradually with the distance from an occupied neighbourhood. The correlation exponent α is the only parameter to be determined by empirical observations.

To discuss the morphology of a system of cities generated in the present model, we show in Fig. 1 our simulations of correlated urban systems for a fixed value of the density gradient λ , and for different degrees of correlation. The correlations have the effect of agglomerating the units around an urban area. In the simulated systems the largest occupation density is situated in the core (which acts as an 'attractive' centre of the city), and this is surrounded by small clusters or 'towns'. (In previous work^{1,3} the compact core has been called the central business district (CBD).) The correlated clusters are fairly compact near their respective centres and become less compact near their boundaries, in qualitative agreement with empirical data on real large cities such as Berlin, Paris and London^{1,13}.

The urban boundary of the largest city is defined to be the perimeter of the connected cluster connected to the CBD. As $p(r)$ decreases as one moves away from the core, the probability that the largest cluster remains connected decreases with r . The mean distance of the perimeter from the centre r_f is determined by the value of r for which $p(r)$ equals the percolation threshold—that is, $p(r_f) = p_c$, so $r_f = \lambda^{-1} \ln(1/p_c)$ (ref. 9).

The urban boundary in the model has the scaling properties of the external perimeter of a correlated percolation cluster in the presence of a gradient. The scaling of the length of the boundary within a region of size l , $L(l) \approx l^{D_e}$, defines the fractal dimension D_e , which we calculate to have values $D_e \approx 1.3$ for the uncorrelated case, and $D_e \approx 1.4$ for strong correlations ($\alpha \rightarrow 0$). Both values are consistent with actual urban data, for which values of D_e between 1.2 and 1.4 are measured¹. Near the frontier and on length scales smaller than the width of the frontier, the largest cluster has fractal dimension $d_f \approx 1.9$, as defined by the 'mass-radius' relation¹², independent of the correlations.

So far, we have argued how correlations between occupancy probabilities can account for the irregular morphology of towns in an urban system. As can be seen in Fig. 2a, the towns surrounding a large city like Berlin are characterized by a wide range of sizes. We are interested in the laws that quantify the town size distribution $N(A)$, where A is the area occupied by a given town, so we calculate the actual distribution of the areas of the urban settlements around Berlin and London. We find (Fig. 3a) that for both cities, $N(A)$ follows a power law.

This result can be understood in the context of our model. The small clusters surrounding the largest cluster are all situated at distances r from the CBD such that $p(r) < p_c$ or $r > r_f$. Therefore, we find $N(A)$, the cumulative area distribution of clusters

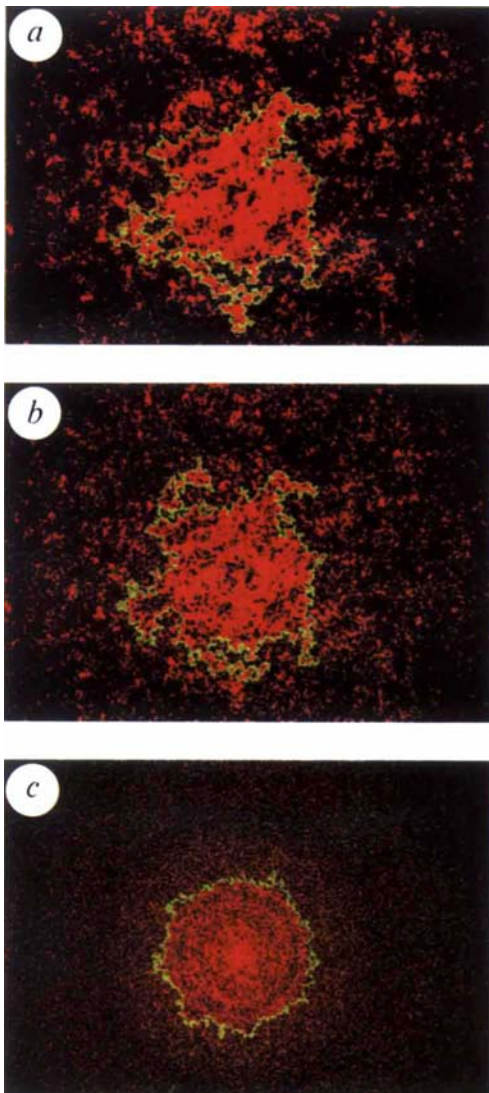


FIG. 1 a–c, Simulations of urban systems for different degrees of correlations. Red indicates the urban areas, and light green shows the external perimeter or urban boundary of the largest cluster connected to the CBD. In all the panels, we fix the value of the density gradient to be $\lambda = 0.009$. a, b, Two different examples of interactive systems of cities for correlation exponents $\alpha = 0.6$ and $\alpha = 1.4$, respectively. The development units are positioned with a probability that decays exponentially with the distance from the core. The units are located not randomly as in percolation, but rather in a correlated fashion depending on the neighbouring occupied areas. The correlations are parametrized by the exponent α . The strongly correlated case corresponds to small α ($\alpha \rightarrow 0$). When $\alpha > d$, where d is the spatial dimension of the substrate lattice ($d = 2$ in our case), we recover the uncorrelated case. Notice the tendency to more compact clusters as we increase the degree of correlations ($\alpha \rightarrow 0$). c, As a zeroth-order approximation, one might consider the morphology predicted in the extreme limit when development units are positioned at random, rather than in the correlated way shown in a and b. The results for this crude approximation of a non-interactive (uncorrelated) system of cities clearly display a drastically different morphology than found from data on real cities (such as shown in Fig. 2a). The non-interactive limit looks unrealistic in comparison with real cities, for the lack of interactions creates an urban area characterized by many small towns spread loosely around the core.

of area A , to be

$$N(A) \equiv \int_0^{p_c} n(A, p) dp \sim A^{-(\tau+1/d_f\nu)} \quad (1)$$

Here, $n(A, p) \sim A^{-\tau} g(A/A_0)$ is defined to be the average number of clusters containing A sites for a given p at a fixed distance r , and $\tau = 1 + 2/d_f$. Here, $A_0(r) \sim |p(r) - p_c|^{-d_f\nu}$ corresponds to the maximum typical area occupied by a cluster situated at a distance r from the CBD, and $g(A/A_0)$ is a scaling function that decays rapidly (exponentially) for $A > A_0$. The exponent $\nu = \nu(\alpha)$ is defined by $\xi(r) \sim |p(r) - p_c|^{-\nu}$, where $\xi(r)$ is the connectedness length that represents the mean linear size of a cluster at a distance $r > r_f$ from the CBD.

In our numerical simulations we find a drastic increase of $\nu(\alpha)$ with the increase of the long-range correlations ($\alpha \rightarrow 0$) (Fig. 3b)⁸. The exponent $\nu(\alpha)$ affects the area distribution of the small clusters around the CBD (Fig. 3c), as specified by equation (1), and can be used to quantify the degree of interaction between the CBD and the small surrounding towns. For instance, for a strongly correlated system of cities characterized by small α , $\nu(\alpha)$ is large so that the area $A_0(r)$ and the linear size $\xi(r)$ of the towns will be large even for towns situated far from the CBD. This effect is observed in the correlated systems of cities of Fig. 1.

In Fig. 3a we also plot the power law for the area distribution predicted by equation (1) along with the real data for Berlin and London. We find that the slopes of the plots for both cities are consistent with the prediction (dashed line) for the case of highly

correlated systems, indicating qualitative agreement between the morphology of actual urban areas and the strongly correlated urban systems obtained in our simulations. Clearly, the exponent of the area distribution provides a stronger test of our model against observations than does the fractal dimension D_c of the perimeter.

We now discuss a generalization of our static model to describe the dynamics of urban growth. Empirical studies¹⁰ of the population density profile of cities show a remarkable pattern of decentralization, which is quantified by the decrease of $\lambda(t)$ with time (see Table 4 in ref. 14). The dynamics in the model (see Fig. 4) are quantified by a decreasing $\lambda(t)$, as occurs in actual urban areas. These considerations are tested in Fig. 2b, which shows our dynamical urban simulations of a strongly interacting system of cities characterized by a correlation exponent $\alpha = 0.05$ for three different values of λ . Qualitative agreement is observed between the morphology of the cities and towns of the actual data of Fig. 2a and the simulations of Fig. 2b.

We wish to comment on the coincidence between the settlement area distribution for different cities and different years (Berlin 1920 and 1945, and London 1981). We first note that these distributions are calculated on length scales larger than the domain of influence of any local planning constraint imposed on the growth of the cities. This fact, together with the fact that interactions among development units can be modelled by a scale-invariant power law, implies that the area distribution $N(A)$ is not affected by the effects of local growth restraints. A more detailed study (for example, calculating $N(A)$ only for those settlements under the domain of influence of applied local

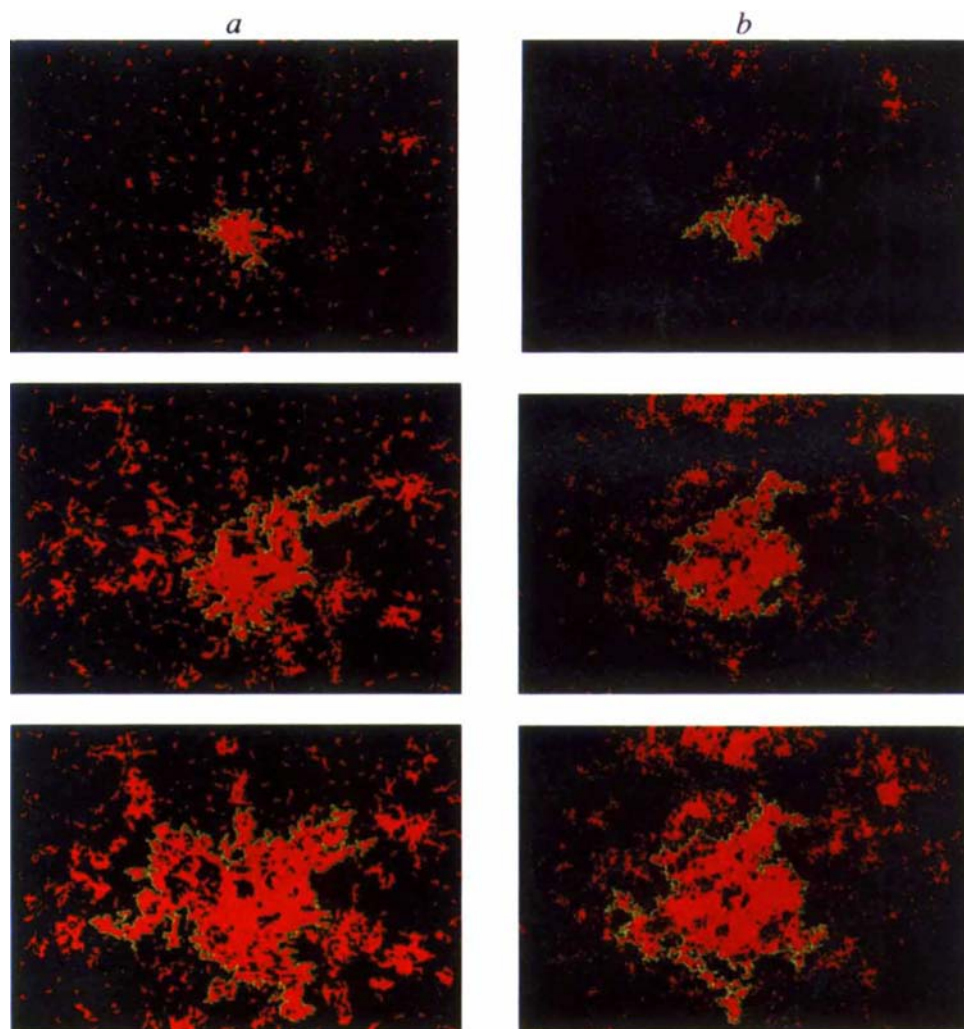


FIG. 2 Qualitative comparison between the actual urban data and the proposed model. *a*, Three steps of the growth with time of Berlin and surrounding towns. Data are shown for the years 1875, 1920 and 1945 (from top to bottom). *b*, Dynamical urban simulations of the proposed model. We fix the value of the correlation exponent to be $\alpha = 0.05$ (strongly correlated case), and choose the occupancy probability $p(r)$ to correspond to the density profiles shown in Fig. 4. We use the same seed for the random-number generator in all panels.

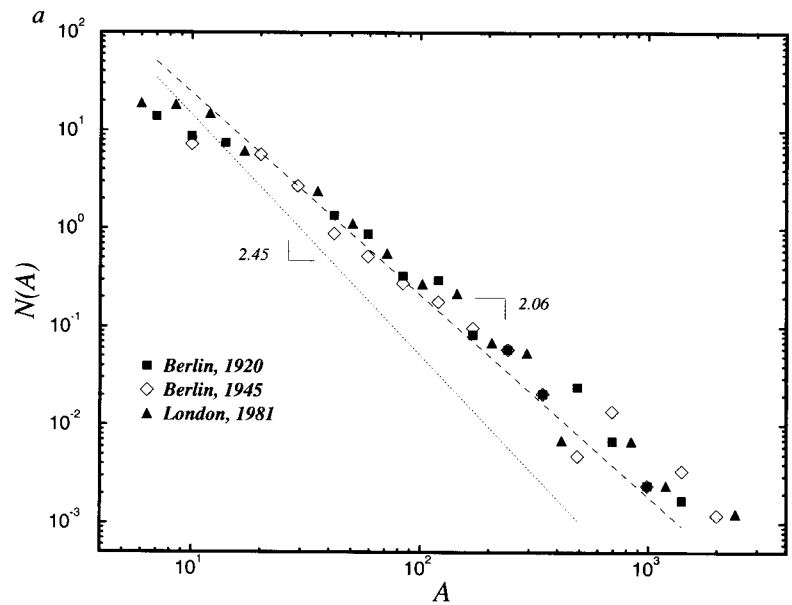


FIG. 3 *a*, Log-log plot of the area distribution $N(A)$ of the actual towns around Berlin and London. We first digitize the empirical data of Fig. 4.1 of ref. 13 (Berlin 1920 and 1945, shown in the last two panels of Fig. 2*a*), and Fig. 10.8 of ref. 1 (London 1981). Then, we count the number of towns that are covered by A sites, putting the result in logarithmically spaced bins (of size 1.2^k , with $k = 1, 2, \dots, 16$), and averaging over the size of the bin. A power law is observed for the area distributions of both urban systems. The dotted line shows the predictions of our model for the uncorrelated case (slope, 2.45), while the dashed line gives results for the strongly correlated case ($\alpha \rightarrow 0$). Note that the area distributions for both cities agree much better with the strongly correlated case ($\alpha \rightarrow 0$). *b*, Connectedness length exponent $\nu(\alpha)$ as a function of the correlation exponent α . *c*, Log-log plot of the area distribution $N(A)$ calculated for the present model for different degrees of correlation. From top to bottom, $\alpha = 0.2$, $\alpha = 0.8$, $\alpha = 1.4$, and uncorrelated case. The linear fits correspond to the predictions of equation (1) using the values of $\nu(\alpha)$ from *b*, and $d_T = 1.9$.

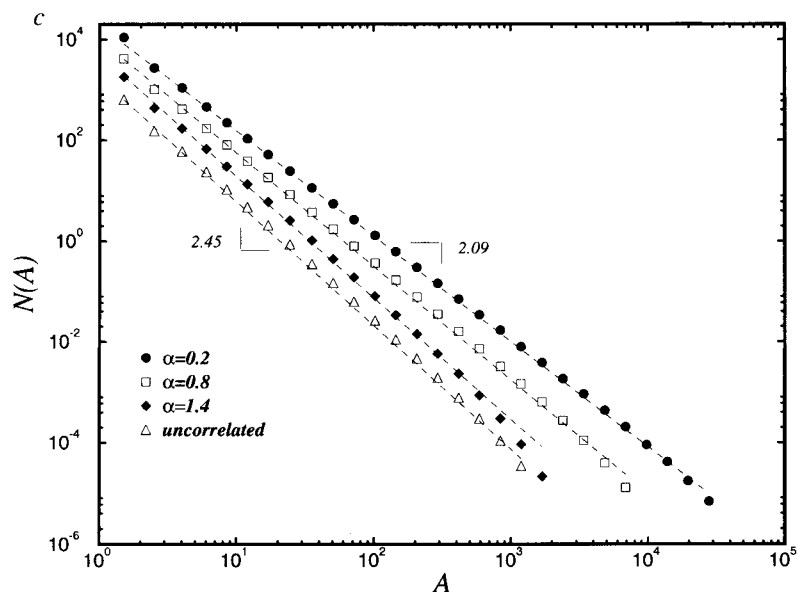
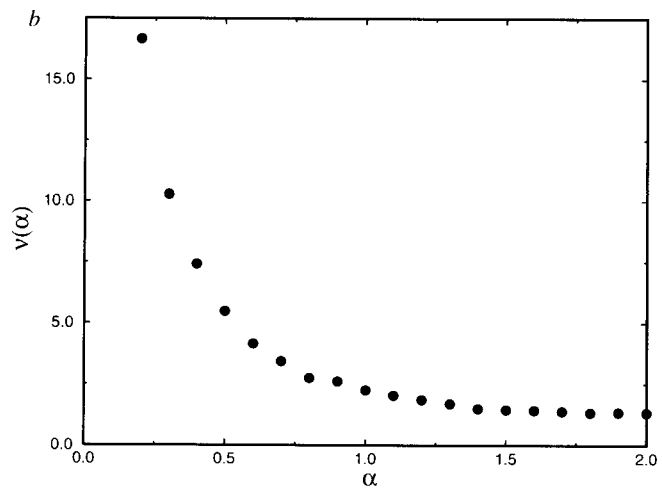
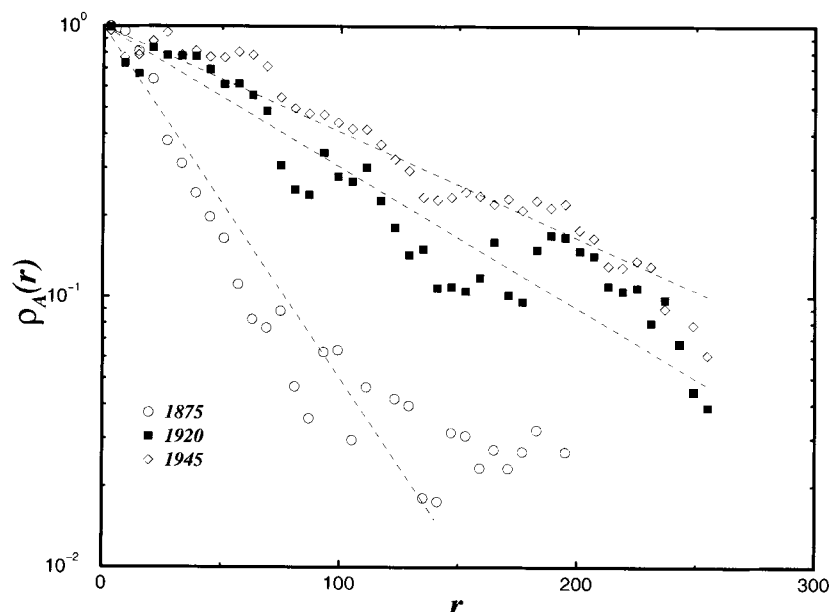


FIG. 4 Semi-log plot of the density of occupied urban areas $\rho_A(r) = e^{-\lambda r}$ for the three different stages in the growth of Berlin shown in Fig. 2a. Least-squares fits yield the results $\lambda \approx 0.030$, $\lambda \approx 0.012$, and $\lambda \approx 0.009$, respectively, showing the decrease of λ with time. We use these density profiles in the dynamical simulations of Fig. 2b. In the context of our model, this flattening pattern can be explained as follows. The model of percolation in a gradient can be related to a dynamical model of units (analogous to the development units in actual cities) diffusing from a central seed or core⁹. In this dynamical system, the units are allowed to diffuse on a two-dimensional lattice by hopping to nearest-neighbour positions. The density of units at the core remains constant: whenever a unit diffuses away from the core, it is replaced by a new unit. The density of units is analogous to $\rho_A(r)$, which in turn is proportional to¹ the population density $\rho(r)$. A diffusion front (defined as the boundary of the cluster of units that is linked to the central core) evolves with time. The diffusion front corresponds to the urban boundary of the central city. The static properties of the diffusion front of this system were found to be the same as those predicted by the gradient percolation model⁹. Moreover, the dynamical model can explain the decrease of $\lambda(t)$ with time that is observed empirically. As the diffusion front situated around r_f moves away from the core, the city grows and the density gradient decreases because $\lambda(t) \propto 1/r_f$.



policies) is needed in order to identify the effects of planning policies on distribution of settlements. Finally, we note that only the parameter λ depends on time; possibly, future urban forms may be predicted by extrapolating λ . □

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Quantification of pre-eruptive exsolved gas contents in silicic magmas

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WATER, carbon dioxide and sulphur are important in the evolution of magmas^{1,2} and the physics of volcanic eruptions^{3,4}. These volatile constituents occur in magmas as dissolved species in silicate melt, but can also form bubbles of exsolved gas if the magma is gas-saturated⁵. Quantifying the total (dissolved plus exsolved) pre-eruptive concentrations of magmatic volatiles is essential for understanding a wide range of magmatic processes. We present a method for quantifying both the amount and distribution of pre-eruptive exsolved gas in a crystallizing silicic magma body. Application to the 0.76-Myr-old⁶ Bishop rhyolitic tuff in eastern

California reveals a pre-eruptive gradient in exsolved gas, with gas contents varying from less than 2 wt% in the deeper regions of the magma body to nearly 6 wt% near the top. This gradient would have promoted stable stratification of the magma body because exsolved gas lowers bulk magma density. More generally, exsolved gas in silicic magmas could contribute to the formation of many hydrothermal ore deposits and to the fluxes of volatile species from volcanic systems.

It has commonly been assumed that silicic magmas only become gas-saturated during shallow ascent and eruption, or during the final pegmatitic stages of plutonic crystallization. But trapped inclusions of gas or fluid in volcanic phenocrysts provide direct evidence that an exsolved gas phase was present during crystallization, even in relatively crystal-poor rhyolitic magmas⁷. These inclusions provide little constraint on the mass fraction of exsolved gas in pre-eruptive magma, but remote-sensing data for volcanic SO₂ emissions suggest that many magma bodies do contain significant amounts of exsolved gas before eruption and that a large proportion of the emitted SO₂ is derived from the pre-eruptive gas phase^{8–11}.

To estimate exsolved magmatic gas contents we first focus on variations in dissolved volatiles and the physical processes by which these variations arise. Glass inclusions in phenocrysts from volcanic tephra provide a record of pre-eruptive conditions because the glass is trapped and quenched magmatic liquid that has retained its original dissolved volatile content¹². We have

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measured pre-eruptive dissolved H₂O and CO₂ contents of rhyolitic melts from the Bishop Tuff by infrared spectroscopic analyses of 150 glass inclusions from early-, middle- and late-erupted magma (Fig. 1). Average dissolved H₂O and CO₂ (± 1 standard deviation) are: 5.6 ± 0.4 wt% H₂O and 60 ± 40 p.p.m. CO₂ (early), 6.0 ± 0.4 wt% H₂O and 120 ± 60 p.p.m. CO₂ (middle), and 4.3 ± 0.3 wt% H₂O and 450 ± 250 p.p.m. CO₂ (late). Corresponding pre-eruptive pressures for each inclusion based on experimentally determined, pressure-dependent solubilities of H₂O and CO₂ vary from 130 to 290 MPa (Fig. 1).

During crystallization of a gas-saturated magma, H₂O and CO₂ are largely excluded from growing crystals, and as a result, additional gas exsolves from the residual melt. This causes the total mass fraction of exsolved gas in a gas-saturated magma to increase during isobaric, closed-system crystallization. Furthermore, CO₂ is much less soluble than H₂O in silicate liquids, so that CO₂ is preferentially partitioned into the coexisting gas phase¹³. Thus, the residual liquids that form during progressive crystallization contain decreasing amounts of dissolved CO₂ and increasing amounts of dissolved H₂O (Fig. 1). The rate of decrease in dissolved CO₂ depends on the amount and composition of the coexisting magmatic gas (Fig. 2). The key to quantifying the amount of exsolved gas is the record preserved in glass inclusions of dissolved CO₂ at various stages of crystallization.

Glass inclusions from individual pumice clasts of the early- and middle-erupted Bishop Tuff generally show negative correlations between uranium and CO₂ contents (Fig. 3). Uranium is an incompatible trace element, whose concentration in the melt increases during crystallization. This inverse relationship is therefore consistent with a gas-saturated system, in which crystallization causes exsolution and consequent decrease in dissolved CO₂. The shallow slopes for early-erupted samples indicate relatively large amounts of pre-eruptive exsolved gas, which tends to buffer melt CO₂ concentrations as crystallization enriches the residual melt in uranium (Fig. 2). The steeper slopes exhibited by the middle-erupted samples indicate that during crystallization, the middle-erupted magma contained less exsolved gas than the early-erupted magma.

We have calculated pre-eruptive exsolved gas contents using glass-inclusion H₂O, CO₂, and trace-element data by assuming that the glass-inclusion compositions are related by fractional crystallization and that crystallization occurred in a closed system, with no gains or losses of crystals or gas. The first assumption is consistent with evidence that fractional crystallization was the dominant evolutionary process in the magma which erupted to form the early and middle portions of the Bishop Tuff^{14, 17}. The second assumption is a reasonable approximation because the crystal contents inferred from trace-element systematics of glass inclusions (9–19 wt%; see Table 1) are within the range of observed crystal contents of pumice from the early and middle parts of the eruption^{16, 18, 19}.

The assumption that there are no gains or losses of gas during crystallization is consistent with the patterns displayed in Fig. 3. As described above, the glass-inclusion data show trends of decreasing dissolved CO₂ with increasing differentiation, as expected for gas-saturated crystallization in a closed system. An alternative possibility, that melts evolved in the presence of a large buffering mass of through-going gas of constant composition, is inconsistent with the large variations in dissolved CO₂ that are observed (Fig. 3 and Table 1). Furthermore, addition of a relatively CO₂-rich gas would result in an increase in dissolved CO₂ with crystallization, contrary to observation, and loss of exsolving gas would yield greater decreases in dissolved CO₂ than are observed. A system that is closed with respect to gas may seem surprising given the density contrast between rhyolitic melt and coexisting, H₂O-rich gas. However, this density contrast is only about 4 times larger than that between quartz and feldspar phenocrysts and the surrounding melt. Phenocryst and glass-inclusion compositions are inconsistent with gravitational settling of crystals in the early- and middle-

erupted parts of the Bishop Tuff magma body^{17, 19}. Gas bubbles with diameters less than about one-half the diameter of the phenocrysts should rise no faster than the crystals sink.

Exsolved gas contents were calculated using the following mass-balance equations:

$$M_{\text{gas}} = \frac{M_{\text{CO}_2}^{\text{bulk}}(1+F) - M_{\text{CO}_2}^{\text{liquid}}}{(1 - M_{\text{H}_2\text{O}}^{\text{gas}})(1+F) - M_{\text{CO}_2}^{\text{liquid}}} \quad (1)$$

and

$$M_{\text{gas}} = \frac{M_{\text{H}_2\text{O}}^{\text{bulk}}(1+F) - M_{\text{H}_2\text{O}}^{\text{liquid}}}{M_{\text{H}_2\text{O}}^{\text{gas}}(1+F) - M_{\text{H}_2\text{O}}^{\text{liquid}}} \quad (2)$$

where M is the mass fraction of the subscripted component in the superscripted phase, liquid refers to H₂O and CO₂ dissolved in the silicate liquid, F is the mass ratio of crystals to melt, and M_{gas} is the total mass of exsolved gas in the system corresponding to the particular value of F . For each pair of inclusions (Table 1), which are taken to represent the initial and final conditions

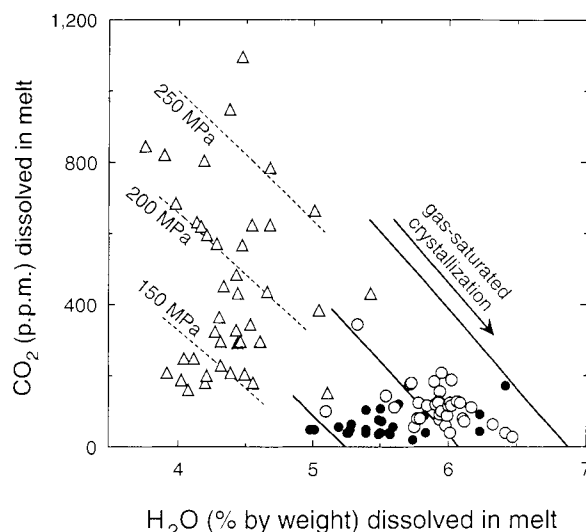


FIG. 1 Dissolved H₂O and CO₂ contents in glass inclusions from quartz phenocrysts of the Bishop Tuff (data from Skirius¹⁸, and P.J.W. and A.T.A., unpublished data). Data are shown for samples from the early (filled circles), middle (open circles), and late (open triangles) parts of the eruption. For clarity, data for the Chidago lobe are not shown. Emplacement of this lobe occurred temporally between the units in our early and middle designations, and the data overlap with both. It is generally inferred, based on geological evidence, that the early-erupted tephra came from near the top of the magma body, and that later-erupted tephra were derived from correspondingly deeper levels¹⁹. Samples were analysed using the methods described in Table 1. Diagonal lines indicate the H₂O and CO₂ contents of rhyolitic melt saturated with H₂O–CO₂ gas at pressures from 150 to 250 MPa and temperatures of 725 °C (solid lines) and 790 °C (dashed lines). These correspond to the pre-eruptive temperatures for the early- and late-erupted magmas, respectively¹⁹. The lines have negative slopes because the amounts of dissolved H₂O and CO₂ are proportional to their fugacities ($f_{\text{H}_2\text{O}}$ and f_{CO_2} , respectively)^{22–24}, and the sum of the partial pressures of these components ($p_{\text{H}_2\text{O}}$ and p_{CO_2}) must equal the total pressure in a gas-saturated melt (f_i is related to p_i by the fugacity coefficient). This assumes that other species (for example, Cl, S) are minor components of the gas and do not change significantly with CO₂/H₂O ratio. Thus at constant total pressure, an increase in $f_{\text{H}_2\text{O}}$ (and hence dissolved H₂O) must result in a decrease in f_{CO_2} and dissolved CO₂ (ref. 22). The arrow indicates the trend of H₂O and CO₂ contents in residual liquids formed by isobaric, gas-saturated crystallization. Textural relations in the late Bishop Tuff samples indicate that inclusions with higher CO₂ contents formed after those with lower values. This suggests that the gas saturation pressures at the high end of the 130–290 MPa range for late Bishop Tuff inclusions are the most representative of pre-eruptive conditions. It also indicates that crystal settling or downward motion were important processes in the crystallization of magma that formed the late Bishop Tuff.

TABLE 1 Analyses of glass inclusions in quartz phenocrysts from the Bishop Tuff

Inclusion	H ₂ O molec. (wt%)	OH ⁻ (wt%)	H ₂ O tot. (wt%)	CO ₂ (p.p.m.)	P _{sat} (MPa)	X _{H₂O} in gas	wt% crystallization	Exsolved gas (wt%)
Blind Spring Hill (early Bishop Tuff)								
2-7.1	3.79	1.71	5.50	109	181	0.94	—	—
2-4.1	3.71	1.58	5.29	64	162	0.96	17 (5)	3.4 (0.8)
3-A5-1	3.66	1.53	5.19	58	156	0.96	—	—
3-A5-2	3.54	1.49	5.02	49	145	0.97	7.0 (1.5)	5.7 (2.3)
5D-L2-2	3.84	1.73	5.57	35	175	0.98	—	—
5D-L1	4.08	1.66	5.74	19	182	0.99	19 (12)	2.0 (1.5)
7B-L1	3.95	1.45	5.39	103	174	0.94	—	—
7B-L2	4.09	1.39	5.48	77	175	0.95	19 (8)	3.8 (1.8)
US Highway 395 (early Bishop Tuff)								
21A-2	4.50	1.33	5.82	90	198	0.95	—	—
21A-L	4.10	1.30	5.39	38	164	0.98	19 (3)	3.0 (0.3)
Chalfant pumice quarry (middle Bishop Tuff)								
35-1-2.1	4.61	1.17	5.78	125	200	0.94	—	—
35-1-3.2	4.66	1.08	5.75	57	189	0.97	10 (5)	1.2 (0.6)
35-4-9.1	4.63	1.27	5.89	182	215	0.91	—	—
35-4-7.1	4.69	1.24	5.93	92	205	0.95	13 (3)	1.6 (0.4)
Chalk bluffs (middle Bishop Tuff)								
18-2.1	4.71	1.24	5.95	208	223	0.90	—	—
18-6.1	4.85	1.25	6.10	82	214	0.96	19 (5)	1.8 (0.5)
Crestview (late Bishop Tuff)								
3-1-13.1	3.12	1.25	4.38	949	262	0.55	51 (10)	0.53 (0.25)

Each pair of inclusions is from an individual pumice clast from the indicated locality. Samples were chosen to represent as much of the eruption duration as possible, and were taken from both pumice fall and unwelded pyroclastic flow deposits. For the early Bishop Tuff, only two inclusions were analysed from each pumice clast; for samples from the middle of the eruption, the pair for which data are given were chosen to represent the endpoints of the trends shown in Fig. 3. Values for molecular H₂O (H₂O molec.) (5,200 cm⁻¹), OH⁻ (4,500 cm⁻¹) and CO₂ (2,350 cm⁻¹) were determined by infrared spectroscopy^{21,22}. The sum of H₂O molec. and OH⁻ is H₂O tot. Analytical uncertainties are ± 0.25 wt% for total H₂O and $\sim 3\%$ of the amount present for CO₂. Data on H₂O and CO₂ for clasts 3-A5, 5D, 7B and 21A are from Skirius¹⁸. Pressures (P_{sat}) at which silicic melt would be saturated with a gas phase containing H₂O and CO₂ were calculated using experimental solubility data²²⁻²⁴. Gas compositions ($X_{\text{H}_2\text{O}}$ in gas) were calculated using a modified Redlich-Kwong equation of state²⁵ for fugacities of pure H₂O and CO₂ and assuming ideal mixing of these components in the gas phase. The weight % crystallization that relates each pair of inclusions is calculated using trace-element compositions (Ca, Mg, La, Ce, Yb, U) of the inclusions as determined by ion-microprobe and bulk distribution coefficients from Lu *et al.*¹⁴ and Cameron¹⁶. The weight % of exsolved gas in the magma at the end of crystallization is calculated as described in the text. Values in parentheses are uncertainties of one standard deviation.

related by crystallization, exsolved gas contents at the beginning ($F=0$) and end of crystallization can be calculated subject to the constraint of constant $M_{\text{H}_2\text{O}}^{\text{bulk}}$ and $M_{\text{CO}_2}^{\text{bulk}}$. This provides statements of equations (1) and (2) for both the initial and final conditions, which can be solved iteratively for the four unknowns ($M_{\text{gas}}^{\text{initial}}$, $M_{\text{gas}}^{\text{final}}$, $M_{\text{H}_2\text{O}}^{\text{bulk}}$ and $M_{\text{CO}_2}^{\text{bulk}}$). Although the gas saturation pressures of inclusions from individual pumice clasts are generally similar (see Table 1), this method does not require isobaric conditions.

Application of this method to the 1991 eruption of Mount Pinatubo (Philippines) yields a pre-eruptive exsolved gas content of 3.6 ± 1.2 wt% (P.J.W., unpublished data). This result is consistent with the 1–6 wt% of exsolved gas estimated by combining

remote-sensing data for SO₂ emissions with experimental constraints on pre-eruptive partitioning of sulphur between rhyolitic melt and coexisting gas^{9,11}. The agreement between these two independent results supports their validity. Unfortunately, in the Pinatubo case both techniques have large uncertainties that are due to the paucity of unleaked glass inclusions in the Pinatubo tephra⁹, to uncertainties in the experimental measurements of sulphur partitioning between melt and gas¹¹, and to uncertainties in the total mass of SO₂ in the eruptive cloud¹¹.

The calculated pre-eruptive exsolved gas contents for the magma body that erupted to form the Bishop Tuff are shown in Fig. 4 as a function of pressure (depth) in the magma body. Our data reveal systematic decreases in exsolved gas content

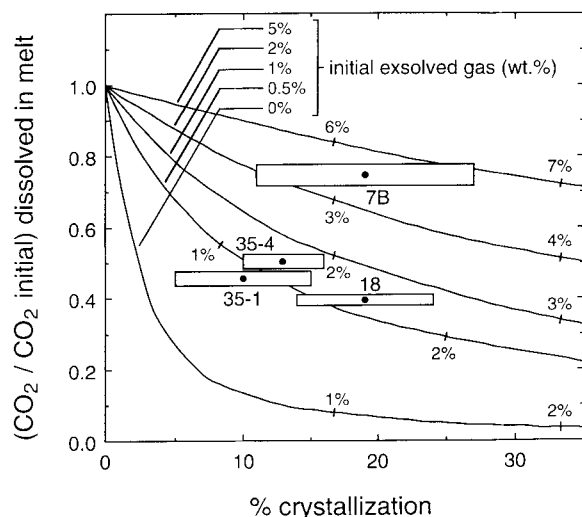


FIG. 2 Effect of crystallization on dissolved CO₂ contents of rhyolitic liquids during isobaric, gas-saturated crystallization in a closed system. Solid curves are for different initial exsolved gas contents and were calculated using solubility and mass-balance constraints for an initial melt with 5.5 wt% dissolved H₂O and 50 p.p.m. CO₂. Tick marks along these curves indicate the percentage by weight of exsolved gas after varying amounts of crystallization. Shown for reference are values corresponding to four pairs of inclusions from Table 1 (filled circles with uncertainty denoted by box), whose exsolved gas contents are closely approximated by the isobaric curves in this diagram. For each pair, the plotted point indicates the ratio of dissolved CO₂ in the second inclusion to that of the first (initial) as a function of the amount of crystallization that relates the two (see Table 1). Other samples from Table 1 are not shown because their particular initial and final H₂O contents and differences in pressure make the isobaric curves depicted here inappropriate for determining the gas content.

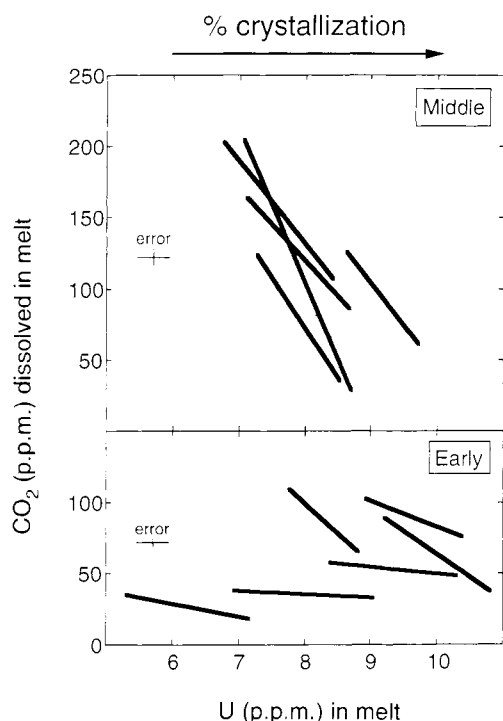


FIG. 3 Variations in uranium and CO_2 contents of glass inclusions from the Bishop Tuff. Solid lines indicate the trend of data from individual pumice clasts. Because uranium is an incompatible trace element, its concentration in residual liquids increases during crystallization. Uranium contents were measured by ion microprobe. Representative error bars are shown for the individual analyses that define the trends. Data for most of the early Bishop Tuff samples are from Lu¹⁷ and Skirius¹⁸. For these samples, only two data points define each line, whereas for samples from the middle part of the eruption, lines are based on data from 4–6 inclusions.

(mass fraction) with pre-eruptive depth. Magma at the roof of the reservoir contained at least 3–4 wt% gas, equivalent to 14–18 vol%, and possibly as much as 6 wt%. Deeper in the reservoir, magma that erupted to form the middle Bishop Tuff contained about 1–3 wt% gas. The exsolved gas content of the late-erupted magma cannot be estimated using the glass-inclusion method because textural complexities and the wide range of calculated saturation pressures for inclusions within individual pumice clasts invalidates the closed-system constraint (see Fig. 1). But if it is assumed that middle-erupted magma evolved by fractional crystallization of melt similar in composition to the most CO_2 -rich inclusions from the late Bishop Tuff^{16,17}, then the pre-eruptive exsolved gas content of the late-erupted magma is constrained by calculation to be ~0.5 wt%.

Exsolved gas has a strong effect on magma bulk density because of its low density relative to silicate liquid and crystals. Therefore, magma bulk densities calculated using the estimated gas contents also vary systematically with pre-eruptive depth (Fig. 4). Our results indicate that exsolved gas variations caused stable density stratification of the magma body, thereby inhibiting convective circulation or overturn, and providing a plausible mechanism for the preservation of vertical compositional gradients in the magma body before eruption.

The upward increase in exsolved gas that characterized the pre-eruptive Bishop magma body (Fig. 4) suggests a process by which gas migrates vertically from lower portions of the body and accumulates near the roof zone. In contrast, the trace-element and CO_2 data for glass inclusions are consistent with closed-system crystallization (Fig. 3). A fundamental paradox of this study is that use of a closed-system constraint in our modelling yields a pattern of pre-eruptive exsolved gas contents that is best explained by open-system processes. If the gradient

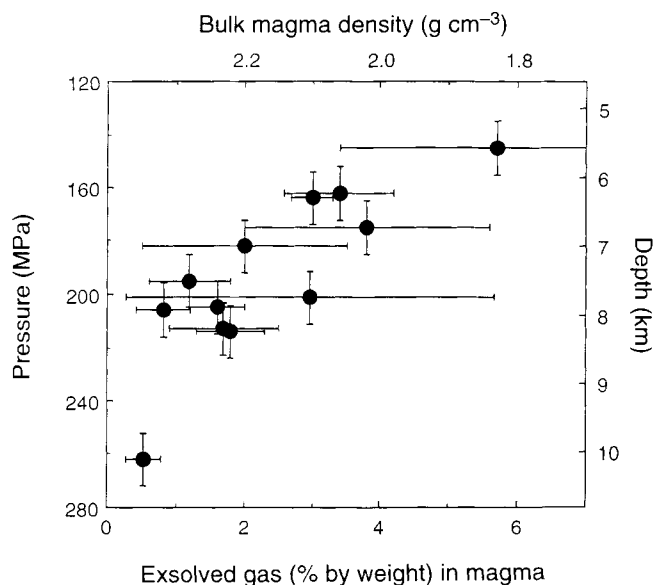


FIG. 4 Exsolved gas contents in the Bishop magma body before eruption as a function of pressure and depth. Data for eight of the samples are listed in Table 1. The remaining values are based on averages of more than two inclusions. The estimate for the late-erupted magma is determined as discussed in the text. Error bars show uncertainty of ± 10 MPa for pressure and the estimated uncertainty in gas contents as reported in Table 1. Bulk magma densities, shown approximately by the scale along the top of the graph, were calculated using partial molar volume data from Lange and Carmichael²⁶, bulk compositions from Skirius¹⁸, average crystal contents and densities from Hildreth¹⁹, and gas densities from a modified Redlich–Kwong equation of state²⁵.

in exsolved gas content formed through open-system processes at an early stage in the development of the magma body, then later closed-system crystallization would have preserved and enhanced this gradient. In his classic work on the Bishop Tuff, Hildreth¹⁹ provided strong evidence that the trace-element zonation of the pre-eruptive magma body existed before crystallization of the phenocrysts. Thus the gradient in exsolved gas may also have existed before crystallization and entrapment of melt inclusions.

The crystallizing Bishop rhyolitic magma evidently contained significant quantities of exsolved gas, especially in the upper region of the magma body where the amount of exsolved gas was comparable to the dissolved volatiles. Although we have provided evidence that large-scale migration of gas did not occur during crystallization, the high exsolved gas content and presumed wide lateral extent at the top of the magma body would have facilitated some mass transfer of magmatic gas (with associated ligands and volatile trace metals) into the overlying hydrothermal system. The amount of exsolved gas inferred for the early-erupted magma is sufficient to have sustained a plinian eruption column³, even without the additional exsolution of dissolved volatiles that would occur during ascent and decompression. Such large amounts of pre-eruptive exsolved gas would also have contained significant quantities of sulphur species, predominantly H_2S (refs 19, 20). Thus the method we have presented for estimating exsolved gas contents could be used to assess the sulphur output from large prehistoric eruptions in order to test theories regarding climate effects of volcanic emissions. □

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A beaked bird from the Jurassic of China

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DISCOVERY of avian remains close to the age of *Archaeopteryx* in the Liaoning Province of northeastern China provides the earliest evidence for a beaked, edentulous bird. The associated wing skeleton retains the primitive pattern found in *Archaeopteryx*, including a manus with unfused carpal elements and long digits. Two leg skeletons from the same site also show an *Archaeopteryx* level of morphology, and provide the earliest indisputable evidence for a covering of body contour feathers. These specimens provide evidence for either an undiscovered pre-*Archaeopteryx* or a rapid, post-*Archaeopteryx* evolution in birds. As the first Jurassic birds to be described from outside Germany, they show that birds with long fingers terminating in large recurved claws were widely distributed. They are not found in the Early Cretaceous sediments of the same region, where there is a diverse assemblage of more advanced flying birds with smaller fingers and claws. The postcranial structure of *Archaeopteryx* and *Confuciusornis* seems to be adapted for climbing tree trunks and may have disappeared near the end of the Jurassic.

Fossils recently described¹ as *Confuciusornis sanctus* include a complete skull and associated wing elements, V10918; a pelvis and leg skeleton, V10895; a second rear leg, V10919; and numerous isolated feathers V10920–V10925 (Fig. 1). *Confuciusornis* shares with *Archaeopteryx*² a primitive avian wing with an unfused carpometacarpus, three unreduced fingers and the general conformation of the humerus. The first phalanx of the first digit is enlarged and the claw is strongly recurved and hypertrophied in comparison to *Archaeopteryx*. Both the first and second digits are much more robust than in *Archaeopteryx*. The hind limbs and pelvis are also very similar to that of *Archaeopteryx*,

and exhibit a robust ischium with an anterodorsal ischial process, as well as a retroverted pubis, missing the distal extremity. They also conform to *Archaeopteryx* in having a long bowed femur. The fibula is present on V10919, where it terminates in an expansion just above the tarsals as in *Archaeopteryx*. The tarsometatarsus appears to be fused proximally, with the third metatarsal being the longest and the fifth metatarsal is present, as it is in *Archaeopteryx*, although it is lost in other birds. The three anterior toes are typically avian, with the middle toe being the longest and exhibiting highly recurved claws, indicative of arboreal habits. The hallux is reflexed, and slightly elevated as in *Archaeopteryx*.

Until the discovery of *Confuciusornis*, there was no direct evidence for a diversification of *Archaeopteryx*-like birds with good climbing ability² but limited power of flight. *Confuciusornis* not only provides evidence for such a radiation but also demonstrates that its members were geographically widespread by the Late Jurassic. This conclusion may be supported by a presumed Jurassic bird from the People's Republic of Korea that has yet to be scientifically described³. A photograph of this partial skeleton has been published and shows the long fingers with big claws characteristic of *Archaeopteryx* and *Confuciusornis*. The Korean specimen is from a region that is close geographically and has a similar stratigraphical section to the region where *Confuciusornis* was found. It is interesting, and we think important, that all of the birds presently thought to be from the Late Jurassic have this type of manus, whereas all of the nearly thirty specimens known from the Early Cretaceous of China and Spain show formation of a carpometacarpus and reduction of the size of the fingers and claws. The Late Jurassic birds are also larger than any of the known Early Cretaceous flying birds, indicating that those birds may have undergone a size reduction at about the same time that they developed improved powers of flight. This may be related to the development of a keeled sternum and the ability to land on and take off from level surfaces. Although the shoulder girdle is presently unknown in *Confuciusornis*, we predict that it will have an ossified sternum with more elongated coracoids than in *Archaeopteryx*. There are typical avian contour body feathers preserved on either side of the tibiotarsus of *Confuciusornis*, about midway along the length, providing new evidence for an early covering of the avian body by contour feathers. This is especially significant because contour feathers are not clearly evident in *Archaeopteryx* and Rubin doubts that it is endothermic⁴.

The skull provides a combination of primitive and advanced characters, strongly resembling the Late Cretaceous *Gobipteryx* in a number of important features. It is badly crushed and some details are obscured. Additional preparation indicates that what was described as an unusually small external nares is an artefact. The skull, like that of *Archaeopteryx*⁵, is of the typical avian, highly modified diapsid type, showing the absence of a post-orbital bone, an enlarged orbit, and a T-shaped lacrimal contacting the jugal ventrally. Unlike *Archaeopteryx*, the antorbital opening is reduced. In general shape, the skull profile conforms to that of *Archaeopteryx*, and Early Cretaceous enantiornithines (*Cathayornis*)⁶ in producing a right-angled triangle in silhouette. It differs from *Archaeopteryx* and closely agrees with the Late Cretaceous Mongolian enantiornithine bird *Gobipteryx* in the following features: (1) large, broad, heavy premaxilla, elongated at the expense of the maxilla, and at its anterior extremity exhibiting numerous pitted foramina associated in modern birds with the passage of sensory nerves, blood vessels and associated structures which are sheltered by the keratinous beak or ramphotheca (earliest evidence for a horny bill in the class Aves); (2) large, conspicuous external narial opening; (3) small antorbital fenestra anterior to the lacrimal; (4) robust, heavy mandible, with anteriorly expanded dentary exhibiting numerous foramina indicating a horny covering over the lower jaw; and (5) pronounced, well developed naso-frontal hinge for a modern prokinetic skull¹.

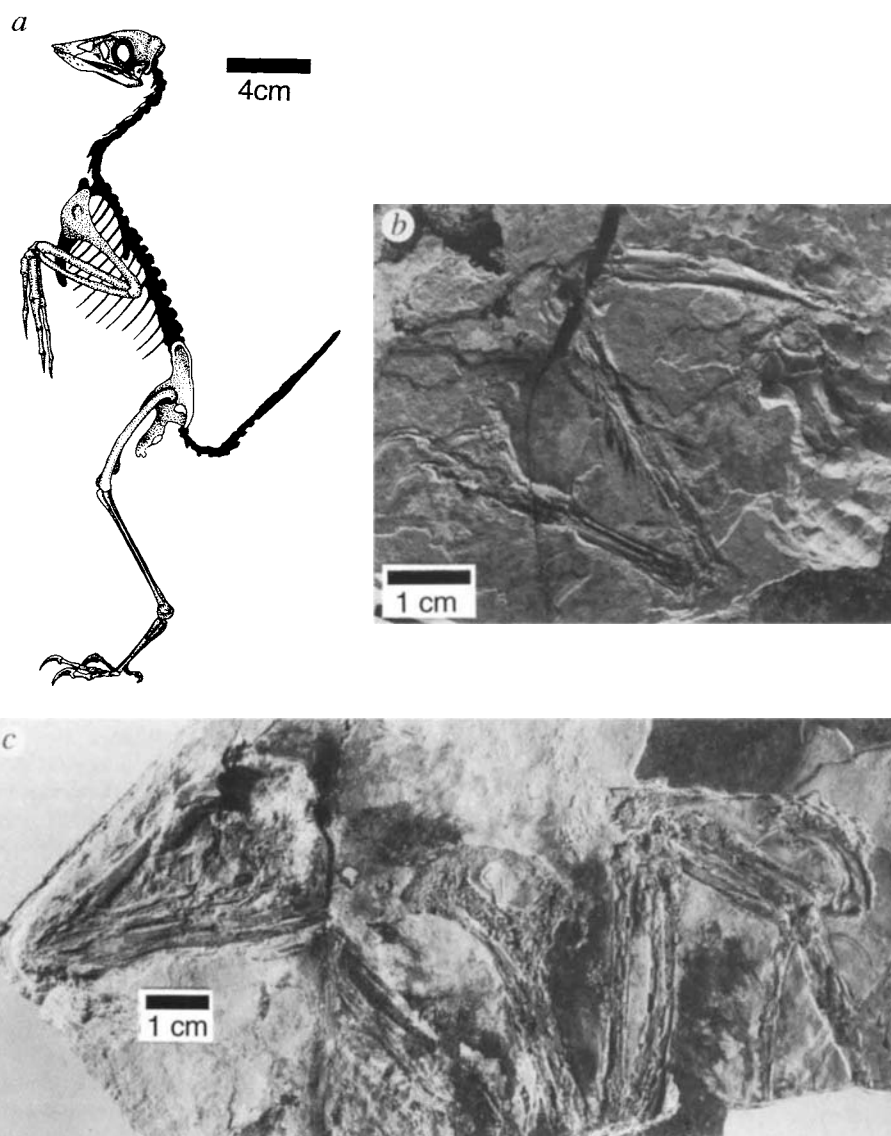


FIG. 1 a, Composite restoration of *Confuciusornis sanctus*. b, Left hind leg, IVPP10895. c, Photograph of the skull and wing skeleton, IVPP10918.

This combination of cranial features is not known in other enantiornithine birds. *Cathayornis* and *Sinornis* have skulls with toothed premaxillaries and maxillaries. In all known Mesozoic ornithurine birds (Hesperornithiformes; Ichthyornithiformes) the horny bill is restricted to the premaxilla. The middle and outer metacarpals are about the same length in *Archaeopteryx*, *Confuciusornis* and *Gobipteryx*, whereas all other Sauriurae have the outer metacarpal elongated distally past the middle metacarpal.

The combination of an advanced skull morphology with an *Archaeopteryx*-like postcranial skeleton may be considered as an example of mosaic evolution. It certainly places *Confuciusornis* on a side branch of avian evolution whose only other known member may be *Gobipteryx* from the Upper Cretaceous of Mongolia (Fig. 2). *Gobipteryx* is an enigmatic Mesozoic bird whose avian status was widely questioned until its affiliation with the Enantiornithes was recognized⁷.

The association of the holotype skull and wing of *Confuciusornis* with the hind limbs is based on their occurrence in the same deposits, their similarity in size, and *Archaeopteryx*-like features that are modified in the numerous specimens of Early Cretaceous birds from the same region.

The femur, tibiotarsus and tarsometatarsus in V10895 are about 3.3, 4.1 and 2.1 cm long, respectively. The tarsometatarsus

in V10915 is 3.2 cm long. From the wing, the first phalanx of digit I is ~1.9 cm and the second metacarpal measures ~2.5 cm. The distal humerus and proximal radius and ulna are missing, so the length of these bones is unknown. The skull measures ~5 cm in total length. The fossil thus compares favourably in size with the smallest (Eichstätt) *Archaeopteryx*, which is ~55% of the size of the London specimen.

The dating of discontinuous lake deposits is difficult, and the Late Jurassic–Early Cretaceous sequence in China is especially controversial. *Confuciusornis sanctus* is from the Yixian Formation that contains volcanic deposits dated by K–Ar at 137 ± 7 Myr and by Rb–Sr at 142.5 ± 4 Myr⁸. Cowie and Bassett⁹ put the Cretaceous–Jurassic boundary at 135 Myr, which would make the Yixian Formation Tithonian, a conclusion that was also reached on ostracods¹⁰. If this assignment is correct, these are the first Jurassic avian remains to be found outside Germany. Some workers use 145 Myr for the boundary¹¹ and controversy over the radiometric age of these deposits continues with the possibility that they may in part be as young as middle Cretaceous. *Confuciusornis* is certainly more primitive and older than the Neocomian birds reported from China, including *Sinornis*¹². All three of the known skeletal specimens show features (unreduced manus, metatarsal V, elongated fibula) that are clearly primitive (shared with *Archaeopteryx*) and different from the

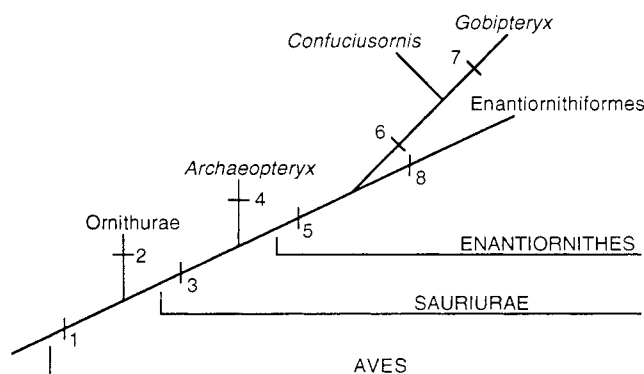


FIG. 2 Cladistic relationships of *Confuciusornis sanctus* to other birds. (1) Feathers, furcula. (2) Distal to proximal metatarsal fusion; distally fused carpometacarpus; cup-shaped scapular facet on coracoid; uncinate processes. (3) Broad furcular arms; anterodorsal ischial process; proximal to distal metatarsal fusion. (4) Reduction of squamosal. (5) Elongated coracoids. (6) Loss of teeth (development of horny bill); small antorbital fenestra; V-shaped lateral groove on ramus. (7) Loss of metatarsal V; reduction of phalanges on the manus digits. (8) Distal elongation of the outer metacarpal.

more derived states found in the Early Cretaceous birds from the same region. It would be as surprising and important to find a Cretaceous bird with unreduced manal digits as it is to find a Jurassic bird.

Archaeopteryx is found in deposits of an arid hypersaline, marine lagoon, but the Chinese bird is from freshwater mudstones deposited in a lake containing ostracods, conchostracans, insects, fishes, amphibians, mammals and plants. This assemblage indicates a surrounding lush forest. The Mesozoic record of avian evolution has been dominated by samples from marine environments. The Chinese records suggest that much of the important early evolution actually took place in the continental interiors. The Liaoning bird exhibits a unique combination of primitive and derived features that associate it, on the one hand, with the *urvogel* *Archaeopteryx*, and on the other with modern birds, and especially with the Late Cretaceous 'opposite bird' or enantiornithine, *Gobiapteryx*^{13,14} (Fig. 2). The Liaoning bird provides evidence for either a long but undiscovered pre-*Archaeopteryx* episode in avian evolution, or for an unexpected, rapid departure from the primitive avian condition to a more derived morphology, including a keratinized beak on the upper and lower jaws. □

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Multiple equilibria in metapopulation dynamics

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THE worldwide loss and fragmentation of natural habitats¹ has led to considerable theory on metapopulation dynamics^{2–8}. One modelling approach, using structured population models^{9,10}, predicts that metapopulations in which local dynamics are affected by migration may have alternative stable equilibria^{11–17}. We have tested this prediction with extensive data on the butterfly *Melitaea cinxia*^{18,19}. Here we show that the probability of local extinction decreases with increasing population size and increasing immigration rate, and that local populations tend to be larger in regions with higher density of extant populations¹⁹, results consistent with model assumptions and predictions^{13,17}. Our results exhibit a bifurcation pattern indicating multiple equilibria¹⁴, with a strikingly bimodal distribution of the fraction of occupied habitat in 65 semi-independent patch networks. These results help to explain observations of species occupying either most, or very little, of the suitable habitat in well-connected patch networks^{14,20,21}. Metapopulations with multiple equilibria may collapse unexpectedly to extinction even in landscapes degrading only slowly. Multiple equilibria make it difficult to predict the occurrence of species in fragmented landscapes.

We have studied the effect of migration on local dynamics and the occurrence of multiple equilibria in a large metapopulation of the Glanville fritillary *Melitaea cinxia* on the Åland islands in southwest Finland^{18,19} (for a map of the study area see ref. 19). In Åland the butterfly breeds on small dry meadows with *Plantago lanceolata*, the larval host plant¹⁸. We have located 1,530 such meadows, ranging from 12 m² to 7.3 ha in size, within an area of 3,500 km². Two surveys revealed 524 and 401 butterfly populations on the meadows in late summer in 1993 and 1994, respectively. Most populations were small, consisting of only a few groups of the gregarious larvae¹⁹. The observed population turnover rate was high, with 256 extinctions and 119 colonizations in one year.

The observed rate of colonization of empty meadows increased with the pooled size of the neighbouring populations (N_{neigh}), reflecting the rate of immigration to the focal meadow, and it was affected by several attributes of patch quality: for example, meadows grazed by cattle or sheep were less likely to become colonized than ungrazed meadows (Table 1). Colonization probability was also affected by the overall trend in the sizes of the neighbouring populations from 1993 to 1994 (N_{trend} ; Table 1). Extinction risk increased with decreasing population size, as expected^{22,23}, but extinction was also affected by N_{neigh} , N_{trend} , patch area and grazing (Table 1).

The effect of N_{trend} on colonizations and extinctions indicates spatially correlated dynamics: neighbouring populations tended to decline, or increase, in synchrony, even though the change was by no means uniform across all of the study area. Two factors are likely to be primarily responsible for the observed spatial correlation: spatially correlated weather effects and parasitoid–host dynamics (G. Lei and I.H., manuscript in preparation).

The risk of local extinction declined with increasing immigration rate, as measured by N_{neigh} (Table 1). We estimated the intrinsic risk of population extinction in the absence of immigration, E_{int} , by setting the value of N_{neigh} to zero in the multiple logistic model (Table 1). E_{int} is substantially greater than E_{obs} , the observed rate of extinctions (Fig. 1). N_{neigh} was about twice

TABLE 1 Multiple logistic regression models for extinction and colonization events in the butterfly *Melitaea cinxia* between 1993 and 1994

Independent variable	Extinctions (n = 253)			Colonizations (n = 114)		
	Coefficient	χ^2	P	Coefficient	χ^2	P
Constant	2.926	28.029	<0.0001	-3.524	31.246	<0.0001
Log (N_{1993})	-2.076	35.974	<0.0001			
N_{neigh}	-0.805	10.965	0.0009	1.076	29.070	<0.0001
N_{trend}	-2.426	74.250	<0.0001	1.474	22.952	<0.0001
Log (area)	-0.666	16.963	<0.0001	0.392	4.122	0.0423
Grazing	1.185	10.572	0.0011	-4.458	22.771	<0.0001
<i>Plantago</i>				0.602	16.803	<0.0001
Drought				-0.833	14.569	0.0001
Grazing $\times$ drought				2.694	17.666	<0.0001
McFadden's R^2		0.246			0.170	
Correctly predicted cases		65% (n = 505)			78% (n = 733)	

Log (area) and log (N_{1993}) are the natural logarithms of patch area and population size (number of larval groups) in 1993; N_{neigh} is a measure of the numbers of butterflies in the neighbourhood of patch i in 1993, calculated as $\log(\sum e^{-d_{ij}} N_j)$, where d_{ij} is the distance between patches i and j (km) and N_j is the size of population j in 1993 (for justification see ref. 30); N_{trend} measures the overall change in the population sizes in the neighbourhood of patch i , calculated as $N_{1994, \text{neigh}} - N_{1993, \text{neigh}}$; grazing indicates whether the patch was actively grazed (1) or not (0); drought (1–3) indicates how much the patch suffered of drought in 1994; and *Plantago* (1–3) measures the abundance of the larval host plant *P. lanceolata* in the patch. Variables with a non-significant effect ($P > 0.05$) were omitted from the model.

as large, on average, for large (≥ 10 larval groups) as for small populations, because of a positive correlation between local population size and the regional density of extant populations¹⁹. The positive correlation between N_{neigh} and population size contributes to the large difference between E_{int} and E_{obs} even in large populations (Fig. 1).

Immigration is expected to reinforce local population sizes and thereby decrease the risk of extinction; this is the 'rescue effect'²⁴. We found that N_{neigh} affected positively the change in the sizes of the persisting populations, although this effect was significant only in the smallest populations, with 1–2 larval groups in 1993. Strong rescue effect is here demonstrated by E_{obs} being smaller than the product $E_{\text{int}}(1 - C)$ (Fig. 1), where C is the probability of colonization of an empty patch (calculated with the model in Table 1).

In the models^{12–14,16,17}, when local dynamics are not influenced by migration, the stable trivial equilibrium corresponding to metapopulation extinction turns to a stable positive equilibrium with increasing colonization rate (see Fig. 3a, b in ref. 14). In contrast, when local dynamics are affected by migration, the trivial equilibrium may bifurcate to alternative stable equilibria, until with even higher colonization rate only the positive equilibrium remains stable (Fig. 2a). Multiple equilibria are generated by the positive feedback between the extent of habitat occupancy (metapopulation size) and local population size.

We tested the predicted bifurcation pattern by dividing the entire material of 524 populations in 1,530 habitat patches into 65 semi-independent patch networks, using criteria explained in Fig. 2b. These networks vary in the number, size distribution and density of habitat patches (Fig. 2b). We used the occupied fraction of the pooled patch area in a network, denoted by p_A , as the dependent variable, and the potential colonization rate,

denoted by M , as the bifurcation parameter. M was calculated as the average patch-specific immigration rate in the network assuming that all patches are occupied (details in Fig. 2b).

The observed relationship between M and p_A (Fig. 2b) greatly resembles the predicted bifurcation pattern (Fig. 2a), with most networks with intermediate M having either a very large or a very small value of p_A . The distribution of the p_A values in the entire material is strikingly bimodal (Fig. 3a), contrasting dramatically with two different null hypotheses (Fig. 3b, c). Such bimodality supports the hypothesis of alternative stable equilibria. The existing metapopulations with less than ~70% of the habitat occupied are predicted to be in transit towards one of the two stable equilibria (Fig. 2b). This prediction is supported by the observed changes in p_A between 1993 and 1994: large (>0.15) absolute changes were mostly observed in networks with M intermediate between 1 and 2 (Fig. 2c; $\chi^2 = 9.40$, $P = 0.002$). Infrequent long-distance migration prevents permanent metapopulation extinction in these semi-isolated patch networks (see map in ref. 19), whereas year-to-year variation in the environmental conditions affecting patch 'carrying capacities' leads to variation in the potential colonization rate (M) and thereby increases the number of metapopulations which spend at least some time in the region of parameter space with multiple equilibria.

These results provide strong support to structured metapopulation models and the predicted multiple equilibria. Our study covered >1,500 habitat patches within a large area (3,500 km²), and there are no reasons to assume that the butterfly is exceptional. Metapopulation models have provided insight to the dynamics of other butterflies²¹, other rare and specialized insects²⁵, and other taxa²⁶. Strong rescue effect and multiple equilibria help to explain previous observations of species occupying

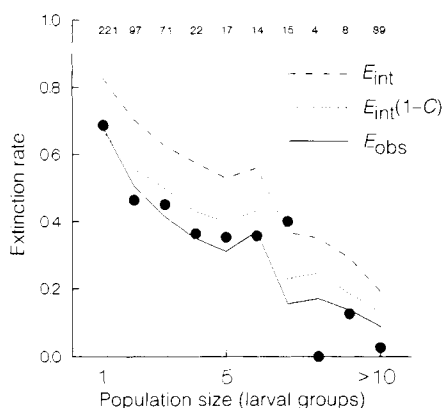


FIG. 1 Rate of population extinction from 1993 to 1994 as a function of population size in 1993, measured as the number of larval groups recorded in the patch. The dots give the observed values, with sample size (number of populations in 1993) given by the small numbers in the upper part of the figure. The continuous line gives the values predicted with the multiple logistic regression model in Table 1, E_{obs} . The broken line gives the intrinsic rate of extinction, E_{int} , calculated with N_{neigh} (effect of immigration) set to zero in the logistic model. The dotted line gives the value of $E_{\text{int}}(1 - C)$, where C , the estimated probability of colonization, was calculated using the model in Table 1.

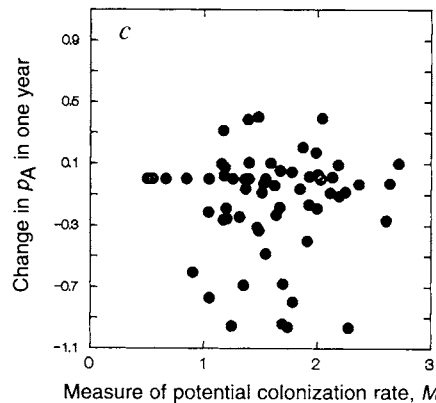
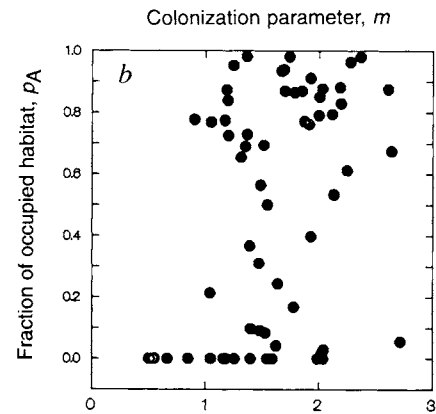
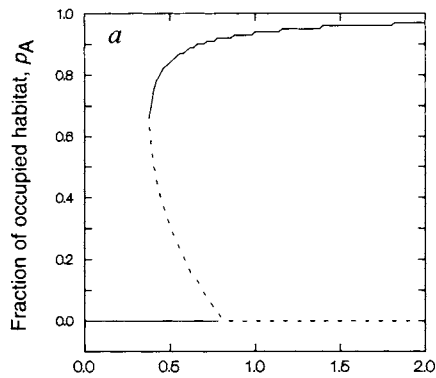


FIG. 2 Bifurcation diagrams for the fraction of occupied habitat (p_A) with increasing colonization rate (m). *a*, A theoretical result based on the model described in ref. 14 (using parameter values in Fig. 3d in ref. 14). Continuous lines represent stable equilibria, broken lines are unstable equilibria. *b*, The empirical result for 65 semi-independent patch networks with at least 5 patches. These networks were delimited in 1993 by T.P. and M.K. using the following criteria: the patches in the network were isolated by at least 1.5 km from any patches in other networks, or by at least 1 km if there was a physical barrier to migration (forest) between the networks³⁰. We have previously shown that ~90% of migrating butterflies move <1.5 km (ref. 18), and that most pairwise distances among patches in different networks are $\gg 1.5$ km, we estimate that two neighbouring networks exchange <1% (usually $\ll 1\%$) of individuals per generation. The networks vary in the number of patches ($n=5-95$), average patch size ($A=0.02-0.56$ ha) and total area covered by the network ($R=1.9-61$ km²). We used the occupied fraction of the pooled patch area ($n \times A$), denoted p_A , as the dependent variable. The potential colonization rate M , which corresponds to the value of the colonization parameter m in *a*, was calculated as $(\sum_i \sum_j e^{-d_{ij}/A_j})^{-1}$, where d_{ij} is the distance between patches i and j (km) and A_j is the area of patch j (ha) (square-root transformation is used to scale patch area to population size³⁰). Note that M is simply the average value of N_{neigh} in Table 1 calculated on the assumption that

all patches are occupied (because M has to measure the potential, not actual, rate of colonization). The square-root transformation is used only to spread the data points more evenly along the x-axis. *c*, The change in the p_A value from 1993 to 1994 as a function of M . Some very large negative changes were observed in networks which had very low density of caterpillars in the patches in 1993.

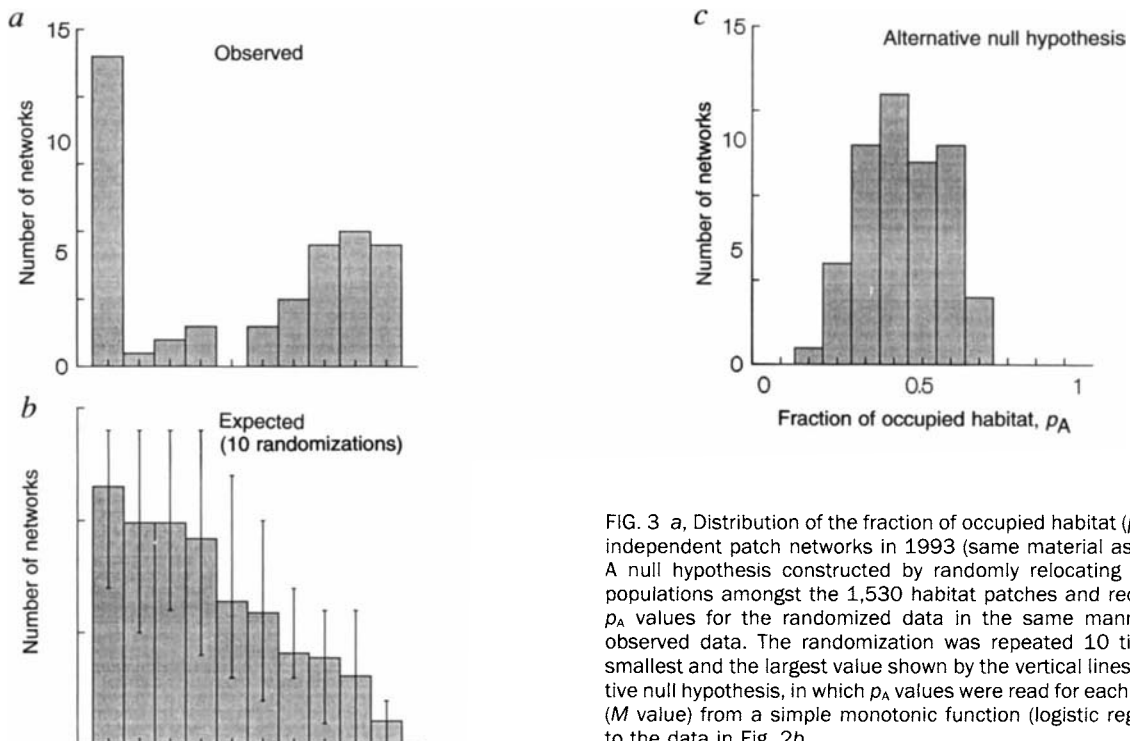


FIG. 3 *a*, Distribution of the fraction of occupied habitat (p_A) in 65 semi-independent patch networks in 1993 (same material as in Fig. 2b). *b*, A null hypothesis constructed by randomly relocating the 524 local populations amongst the 1,530 habitat patches and recalculating the p_A values for the randomized data in the same manner as for the observed data. The randomization was repeated 10 times, with the smallest and the largest value shown by the vertical lines. *c*, An alternative null hypothesis, in which p_A values were read for each patch network (M value) from a simple monotonic function (logistic regression) fitted to the data in Fig. 2b.

either most, or none, or only little, of suitable habitat in well-connected patch networks^{14,20,21,27}. Some might argue that a metapopulation in such a network is really just a single population, but this ignores that the 'population' is structured into numerous extinction-prone units, that most of the butterflies stay in their natal patch (~80% in the case of *M. cinxia*), and that migration, when it occurs, is strongly distance dependent¹⁸. Structured metapopulation models provide a causal explanation of the inferred multiple equilibria (Figs 2b and 3a), which could only be attributed to an unknown 'Allee effect' if the metapopulation would be perceived as a single panmictic population. The occurrence of multiple equilibria in metapopulation dynamics has important implications for conservation. Metapopulations with multiple equilibria may unexpectedly become extinct even in stationary environments. The recent widespread and rapid decline of butterflies in northern Europe^{21,28,29} might have been accelerated by such highly nonlinear metapopulation dynamics. Any attempts to re-establish a metapopulation into an empty patch network should take into account the possibility of multiple equilibria. Finally, multiple equilibria impose a constraint on our ability to predict the occurrence of species in fragmented landscapes. □

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Predation risk and the cost of being fat

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SMALL birds increase their fat reserves in winter as insurance against reduced or unpredictable food supplies¹: fat is accumulated daily from feeding and utilized overnight². Field observations indicate that birds often maintain smaller reserves than expected², which implies that there is a cost of being fat³. One such cost could be that an increased fat load reduces manoeuvrability, thus increasing the risk of predation^{3,4}. Here we demonstrate a link between fat reserves and predation risk by describing changes in body mass (roughly equivalent to fat reserves) that have occurred in British populations of the great tit, *Parus major* since 1950, a period when the numbers of its principal predator, the sparrowhawk *Accipiter nisus*, changed markedly. Furthermore, these changes resulted from individual tits adjusting their mass, rather than from the selection of heavier great tits by hawks.

The great tit is a small (mass ~18 g) bird, common throughout Europe in a variety of habitats⁵. In Britain, its principal predator is the sparrowhawk^{5,7}, which was widespread and abundant until the late 1950s, when its population was severely reduced by organochlorine pesticide poisoning^{8,9}. By 1960, the sparrowhawk was absent from much of eastern and central England where pesticide use had been most intense, although healthy populations remained in Wales and southwest England⁸. Following regulation of these pesticides during the 1960s, sparrowhawks increased rapidly, recolonizing many central counties during the 1970s and eastern ones during the 1980s^{8,9}.

One of the most intensive and longest-running population studies of the great tit is that in Wytham Woods, Oxfordshire, carried out by the Edward Grey Institute^{5,6}. Although breeding

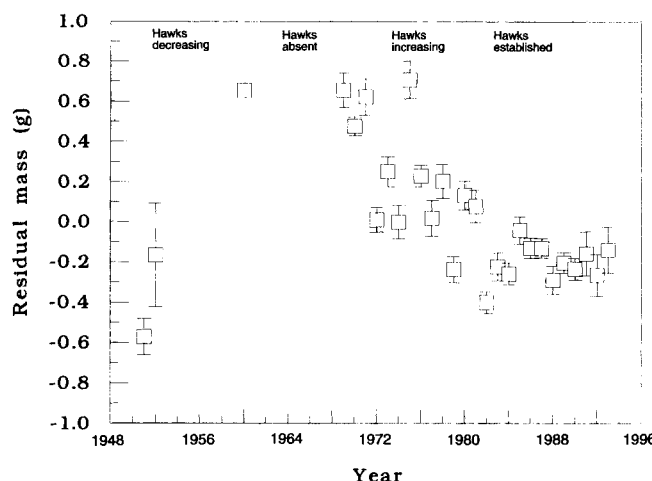


FIG. 1 Changes in winter (October–March) great-tit mass in Wytham Woods between 1951 and 1993 (mean $\pm$ 1 s.e.). Mass was corrected by regression for body size, time of day, and ambient temperature (mean temperature at Oxford on the day of capture) by using the equation: $\text{mass} = -22.7 + 0.264 \text{ wing length} + 1.43 \text{ time} - 0.389 \text{ temperature}$ ($F_{1,5091} = 815.4$, $P < 10^{-6}$), which explained 32.5% of the original variance. Repeat measurements of an individual within a winter were averaged (as were their predictors) so that each bird is represented once only. All Wytham analyses are therefore based on 5,194 observations of which exactly 100 were made before 1965. Analyses were initially performed on the sex and age classes separately; as no significant differences or interactions were found between them, sex and age classes were pooled. The negative trend since 1969 was highly significant (1969–1993: residual mass = $2.66 - 0.0326 \text{ year}$, $F_{1,23} = 32.0$, $P < 10^{-6}$, where year 1901 is 1, and so on). From the uncorrected mass data (which cover more years in the 1950s and 1960s), the mean of year means of 1960–1972 ($19.65 \pm 0.305 \text{ s.d.}$ ($n = 8$)) differed significantly from that of 1950–1959 (18.79 ± 0.711 ($n = 6$, $F_{1,12} = 9.65$, $P = 0.009$)) and of 1973–1980 ($19.18 \pm 0.181 \text{ s.d.}$ ($n = 8$, $F_{1,14} = 14.37$, $P = 0.002$)), indicating that the tits were consistently heavier during the 1960s. The mean of 1981–1993 (18.84 ± 0.164 ($n = 13$)) also differed significantly from the 1973–1980 period ($F_{1,19} = 19.43$, $P < 0.0003$). Great tits were markedly heavier during the years when sparrowhawks were absent, and declined in mass after their return.

TABLE 1 Residual mass of great tits

Period	Wytham Woods, Oxfordshire Beech		Region	Wales and southern England Habitat	
	No mast	Mast		Other	Homestead
1960–1972 (hawks absent)	0.367 ± 0.9403 ($n=392$)	0.476 ± 0.9537 ($n=403$)	Western (hawks present)	0.1069 ± 0.9400 ($n=250$)	0.0304 ± 0.9077 ($n=339$)
1973–1980 (hawks returning)	0.264 ± 1.0316 ($n=571$)	0.081 ± 0.9521 ($n=778$)	Central (hawks returning)	-0.0719 ± 0.9613 ($n=251$)	0.1567 ± 1.0186 ($n=1,091$)
1981–1993 (hawks established)	-0.125 ± 1.0224 ($n=1,481$)	-0.247 ± 0.9127 ($n=1,470$)	Eastern (hawks absent)	-0.2697 ± 0.9764 ($n=1,199$)	0.0563 ± 1.0279 ($n=1,322$)

Data (mean $\pm$ s.d.) for Wytham are divided according to the beechmast crop and/or sparrowhawks; national data are divided by sparrowhawk regions and by habitats. In Wytham, during the re-establishment of the hawk population (1972–1980), great tits were heavier when mast was absent (one-way ANOVA, $F_{1,1347}=11.4$, $P=9 \times 10^{-4}$); this has continued subsequently² (1981–1993: $F_{1,2949}=11.76$, $P=6 \times 10^{-4}$). Beechmast had no effect when sparrowhawks were absent. This difference in response of mass to a mast crop in the presence or absence of sparrowhawks was itself significant in a two-way ANOVA (interaction of beech and sparrowhawk presence, $F_{2,5089}=6.03$, $P=0.002$). Nationally the habitat effect on mass differed by regions. In the west, great tits were marginally (but not significantly) lighter at homestead sites but were significantly heavier at homesteads in the absence of sparrowhawks (central: $F_{1,1340}=10.5$, $P=9 \times 10^{-4}$; eastern: $F_{1,2519}=66.3$, $P<10^{-6}$). The interaction between habitats and regions was significant ($F_{2,4446}=9.63$, $P=7 \times 10^{-5}$). Habitat differences were maintained if variation between years was removed by ANOVA, but now the difference in the west was significant also (west: $F_{1,589}=4.6$, $P=0.032$; central: $F_{1,1340}=7.4$, $P=0.007$; east: $F_{1,2519}=58.5$, $P<10^{-6}$). Mass in the central region responded to habitat during the hawks' return in the same way as in the eastern region without hawks. This may appear to conflict with the Wytham data. However, because we expect sparrowhawks to have occupied optimal habitat (woodland) before secondary habitats (homesteads) during their advance, it is consistent that great tits in homesteads would remain unaffected by sparrowhawk predation longer than those in woodlands (such as Wytham).

until the 1950s, sparrowhawks failed to breed in Wytham from 1960 until 1973, when one pair bred. There were few winter records at this time¹⁰. Since 1978, 6–8 pairs have bred¹⁰; the winter population is about 25 birds¹¹.

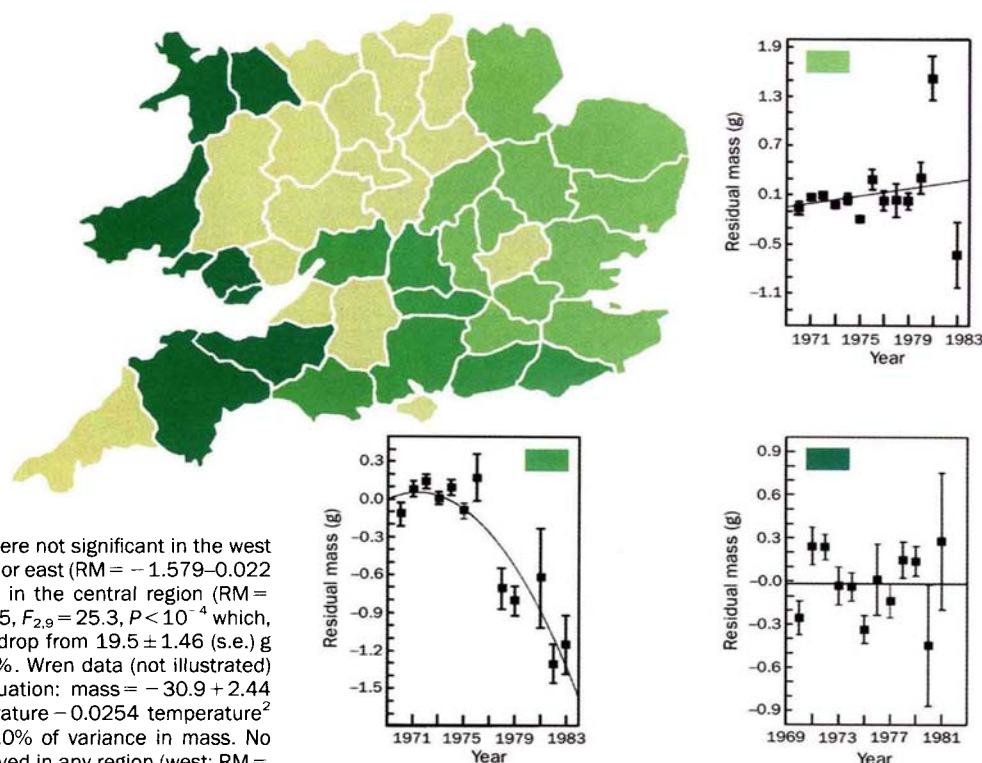
Winter trapping of great tits in Wytham began in 1947. Although few measurements were taken before 1970, about 8,500 winter weights were recorded (to 0.1 g), together with the time at weighing, which is important because mass increases through the day with increasing fat reserves². Wing length was often also recorded as a measure of body size^{12–15}. When corrected for body size, the residual variance in mass largely reflects variation in fat reserves^{1,2,16}. We also made a correction for

ambient temperature because mass increases with declining temperature owing to elevated fat levels², which is itself evidence that the birds' mass is not normally constrained by deteriorating conditions. Thus 'mass' refers to residual mass after making these corrections. In this report we compare the mass of great tits from Wytham before the sparrowhawks disappeared, during their absence, and after their return. If the tits lower their mass in the presence of a predator, we might expect it to increase in the predator's absence.

In Wytham, great tits weighed less before the decline in sparrowhawk numbers and significantly more in the late 1960s and early 1970s (when the sparrowhawk was absent) than in the late

FIG. 2 Changes in winter (October–March) great-tit mass across 26 counties of Wales and England (data from the British Trust for Ornithology). Mass was corrected by regression for body size, time of day, and ambient temperature (mean temperature for the month of observation, from the nearest meteorological station, or averaged for the county centre from several stations in neighbouring counties) (by using the equation: $\text{mass} = 8.18 + 0.227 \text{ wing length} + 1.43 \text{ time} - 0.612 \text{ temperature}$ ($F_{1,4609}=525.1$, $P<10^{-6}$), which explained 25.5% of the variance in mass. Only measurements taken at the first trapping of an individual were used. Counties are grouped into three regions: west (sparrowhawks remained), central (sparrowhawks returned in 1970s), and east (no sparrowhawk return by 1980). Counties with insufficient data are left white. Wytham is marked 'W' but Wytham data (Fig. 1) were not included here.

Regressions of mass (RM) on year were not significant in the west ($\text{RM} = -0.015 - 0.000 \text{ year}$, $r^2 = 0.00$, NS) or east ($\text{RM} = -1.579 - 0.022 \text{ year}$, $r^2 = 0.04$, NS) but highly significant in the central region ($\text{RM} = -51.81 + 1.45 \text{ year} - 0.01 \text{ year}^2$, $r^2 = 0.85$, $F_{2,9}=25.3$, $P<10^{-4}$ which, from the uncorrected data, represents a drop from 19.5 ± 1.46 (s.e.) g in 1972 to 17.6 ± 0.40 g in 1983, or 9.7%. Wren data (not illustrated) were corrected using the regression equation: $\text{mass} = -30.9 + 2.44 \text{ wing length} + 0.792 \text{ time} + 0.375 \text{ temperature} - 0.0254 \text{ temperature}^2$ ($F_{4,3673}=494.8$, $P<10^{-6}$), explaining 35.0% of variance in mass. No change in wren mass with time was observed in any region (west: $\text{RM} = -4.47 + 0.0581 \text{ year}$, $F_{1,10}=2.84$; central: $\text{RM} = 1.15 - 0.015 \text{ year}$, $F_{1,10}=0.60$; east: $\text{RM} = 0.18 - 0.0006 \text{ year}$, $F_{1,12}=0.00$).



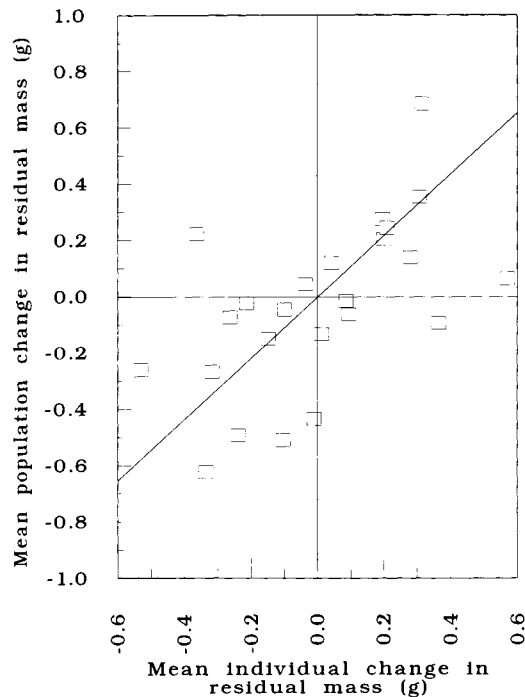


FIG. 3 The relationship between mean change between years in residual mass for the Wytham population and the change within re-trapped individuals. Residual mass was corrected for age effects before calculating the mean change in either dataset. Population and individual changes were highly correlated ($r_{22}=0.57$, $P=0.004$). The reduced major axis regression slope (mean population change = $1.108 \times$ mean individual change ± 0.1942 s.e.) shows that changes in the population can be accounted for by changes within individuals as the population change was only 1.1 times that found within individuals (approximate 95% confidence limits: 0.7–1.4 times). There is therefore little evidence that the reduction in great-tit mass in the presence of sparrowhawks was due to selection of heavier individuals by the hawks. It might be argued that the reduction in mass reflects a movement of tits out of optimal feeding sites under pressure from sparrowhawks, or that, in the presence of hawks, the greater time required by tits for vigilance reduced available foraging time. As these arguments require that the reduced mass reflects a constraint to their ability to fatten further, they contradict the facts that, in the presence of hawks, tits are lighter when food is more abundant² (Table 1), and that socially subordinate tits, which generally face the greatest exposure to predation^{19,20}, are fatter than dominants^{2,21}. Our observations are consistent, however, with the suggestion that, because sparrowhawks take prey by surprise but just after the prey has taken wing, an additional fat load carries a predation cost against which starvation risk must be balanced.

1970s and subsequent decade. Between 1970 and 1980, mass decreased by about 0.9 g. (Fig. 1). This pattern was repeated nationally. Between 1970 and 1983, a further 4,614 records of great tit mass were archived at the British Trust for Ornithology. These data cover 26 counties (Fig. 2), which can be classified⁸ according to whether the sparrowhawk population was largely unaffected by pesticides (596 observations from western Britain), returned during the 1970s (1,738 observations from central southern England), or had not returned by 1980 (2,280 observations from eastern England). Further, 3,678 observations of wrens, *Troglodytes troglodytes*, were reported, allowing comparison with a small passerine rarely taken by sparrowhawks⁸ (329 west, 846 central, 2,503 east). In the west and east regions no significant change in great tit mass occurred (Fig. 2), but in central England, where sparrowhawks increased, great-tit mass dropped considerably. Wrens showed no significant change in mass over time in any region, as expected for a species rarely preyed by sparrowhawks.

Beechmast, *Fagus sylvatica*, is an important winter food of the great tit, but its abundance varies greatly between winters. In the presence of sparrowhawks, Wytham great tits are leaner if beechmast is abundant, suggesting that when food is plentiful the reduced risk of starvation allows a lowering of reserves². When sparrowhawks were absent from Wytham, however, great-tit mass was unaffected by beechmast (Table 1). Nationally, a broad classification of habitats into 'homestead', where food was provided, and 'other' (largely natural or semi-natural sites)

allows a comparison of mass across the three regions. Great tits in the west (sparrowhawks present) weighed less at homestead sites (Table 1), but they were heavier at these sites in the central and eastern regions (sparrowhawks absent).

These analyses provide strong evidence that predation risk is a major cost determining the optimal fat levels carried by small birds. In Wytham, great tits reduced their mass in the presence of sparrowhawks (temporal control), as did tits across southern England as sparrowhawks returned (geographical control). This was not so in a species rarely taken by sparrowhawks (species control). In the presence of sparrowhawks, great tits weighed less when food was abundant or predictable (beechmast present or homesteads), suggesting a predation cost. In the absence of sparrowhawks, the tits weighed more and at homestead sites were significantly heavier, suggesting that human contributions to their food may have been significant.

The decline in mass could result from the direct effects of selection by sparrowhawks or from birds reducing fat loads in response to increasing sparrowhawk numbers (not mutually exclusive hypotheses). Between 1969 and 1993 in Wytham, 90% of the annual change in population mean mass was due to adjustments made by individuals (Fig. 3). Such large adjustments could not result from changes in any tissue other than fat reserves². Because the heritability (which sets an upper limit to the response to selection¹⁷) of fat reserves is negligible¹⁸, we argue that these responses to predation resulted not from selection, but from adjustment of fat by the tits according to a trade-off between the risks of starvation and predation. □

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Asymmetric segregation of Numb and Prospero during cell division

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A CELL can divide asymmetrically by specifically segregating a determinant into one of its daughter cells¹. The Numb protein is a candidate for such a determinant in the asymmetric cell divisions of the developing *Drosophila* nervous system^{2,3}. Numb is a membrane-associated protein that localizes asymmetrically during cell division and segregates into one daughter cell, where it is required for the specification of the correct cell fate³⁻⁵. Here we show that a nuclear protein, Prospero^{6,7}, translocates to the membrane at the beginning of cell division and colocalizes with Numb throughout mitosis, suggesting a common mechanism for asymmetric segregation. Numb and Prospero localization is coupled to mitosis and tightly correlated with the position of one of the two centrosomes. In contrast to centrosome positioning, however, Numb and Prospero localization is independent of microtubules. Cytochalasin D treatment suggests that the process is also independent of actin. We propose that there is an organizer of asymmetric cell division which provides positional information for both the orientation of the mitotic spindle and asymmetric localization of Numb and Prospero.

Localization of Numb during mitosis was studied in developing neuroblasts (Fig. 1). During interphase and early prophase, Numb is homogeneously distributed along the cell membrane (Fig. 1a), but starting in late prophase, Numb forms a crescent overlying one of the two centrosomes. In all metaphase cells the protein is localized in a tight crescent (Fig. 1b). The localization is maintained through anaphase (Fig. 1c), and in telophase Numb segregates preferentially into one of the two daughter cells (Fig. 1d), where it is found evenly distributed along the membrane shortly after mitosis. In *string* mutant embryos, where cells arrest in the G2 phase of the cell cycle^{8,9} and do not enter mitosis, Numb remains evenly distributed along the cell membrane (data not shown), suggesting that localization is dependent on entry into mitosis.

Asymmetric localization of Prospero, a homeodomain-containing protein involved in neural precursor development^{6,7}, has been observed in neuroblasts¹⁰ and in dividing sensory organ precursor (SOP) cells (Fig. 2). Before cell division, *prospero* RNA, but not protein, is found in SOP cells⁶. We first detect the protein in the cytoplasm during prophase (Fig. 2a). Shortly afterwards it translocates to the membrane and colocalizes with Numb (Fig. 2b, h). From this time on through to telophase, the distributions of the two proteins are identical. They localize asymmetrically at the same time (Fig. 2c, i) and segregate into the same daughter cell, where Prospero is released from the membrane and transported into the nucleus (Fig. 2d) while Numb remains associated with the plasma membrane (Fig. 2j). Numb and Prospero localization are independent of each other: *numb* mutants show no defects in Prospero localization (Fig. 2e, k) and *prospero* mutants show no defects in Numb localization (Fig. 2f, l). Their striking colocalization, however, suggests a common mechanism for asymmetric segregation.

Both the Numb and Prospero crescents always overlie one of the two centrosomes, suggesting a functional connection between these protein localization events and the position of the centrosome. This possibility was tested in *pebble* mutant embryos, in which mitosis occurs without cytokinesis^{11,12}, giving rise to cells with two nuclei and four centrosomes (Fig. 3). These centrosomes can either form two pairs on opposite sides of the

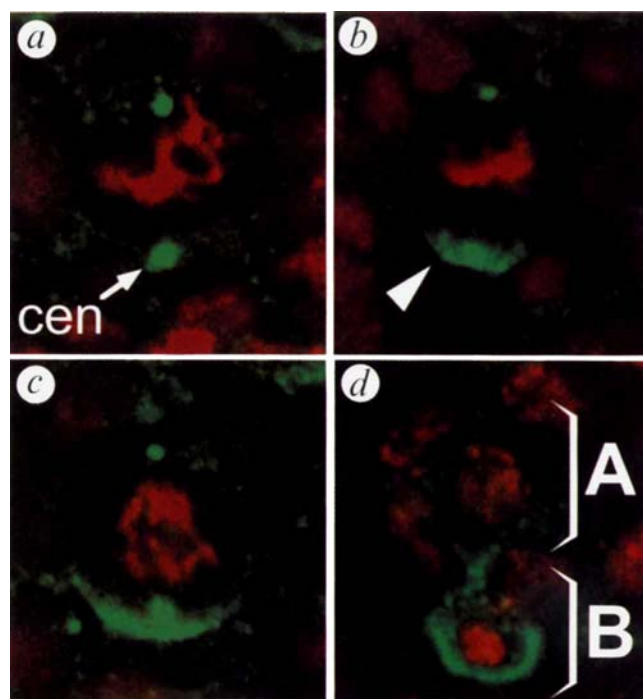


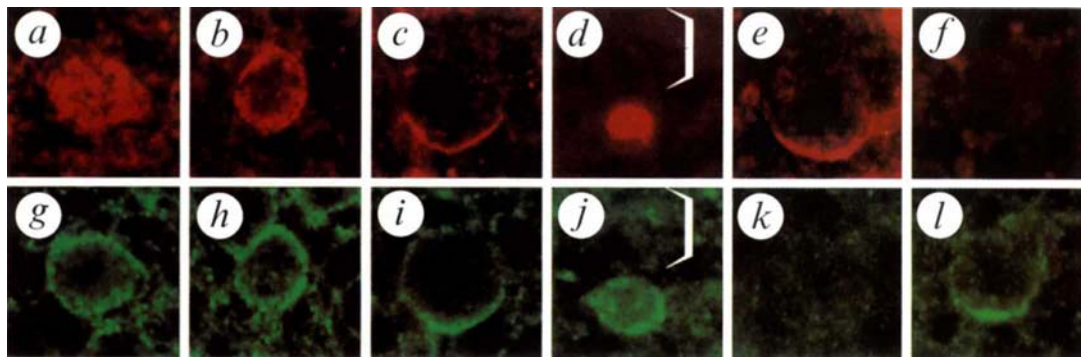
FIG. 1 Numb localization during mitosis. In all interphase cells, the Numb protein is evenly distributed along the cell membrane. During mitosis, asymmetric distribution of the Numb protein is observed in the asymmetrically dividing neuroblasts of the central nervous system (CNS), in the sensory organ precursor (SOP) cells of the peripheral nervous system (PNS) and in the interstitial cell precursors (ICP) of the developing midgut²⁴ but not in the symmetrically dividing cells of the epidermis (data not shown). a–d, Neuroblasts in consecutive phases of mitosis. Centrosomes (cen, green), DNA (red) and the Numb protein (green) are shown. a, Early prophase: the chromosomes have condensed, the centrosomes have migrated to the opposite poles of the cell, but no signs of asymmetric Numb localization are visible. b, Metaphase: the chromosomes are aligned in the metaphase plate and Numb is localized (arrowhead) to the membrane area overlying one of the two centrosomes. c, Anaphase: the chromosomes migrate to the opposite poles. d, Telophase: cytokinesis separates the dividing cell into a smaller ganglion mother cell (B) and a larger neuroblast (A)²⁵. Numb segregates into the ganglion mother cell. Both centrosomes are located outside this focal plane and are not visible.

METHODS. Stage-10 *Drosophila* embryos were fixed and stained for immunofluorescence as described³. Images were recorded with a Bio-Rad confocal microscope. Rabbit antibodies against Numb³ and γ -tubulin (B. Oakley and Y. Zheng, manuscript in preparation) were mixed and detected simultaneously. The specificity of the two individual antibodies was determined in a separate experiment (data not shown). Propidium iodide was used to visualize mitotic chromosomes²⁶.

cell (Fig. 3b) or be more widely separated from each other (Fig. 3c, d). In either case, the Numb crescent remains associated with two of the four centrosomes. When two pairs of centrosomes are visible, Numb localizes into a tight crescent overlying one of these pairs (Fig. 3b). When four individual centrosomes are visible, Numb localizes into a crescent that is centred between two of the four centrosomes (Fig. 3c) or forms two individual crescents overlying two of the four centrosomes (Fig. 3d). A similar 'double crescent' is observed for Prospero (data not shown). These results suggest that the positioning of the centrosome and asymmetric localization of Numb and Prospero are highly coordinated during mitosis.

Centrosome localization does not require Numb or Prospero (data not shown). To determine whether localization of Numb and Prospero depends on correct localization of the centrosomes, we made use of the fact that centrosome localization is microtubule-dependent^{13–15} (Fig. 4a–e). After destruction of microtubules with colcemid, cells fail to form a functional

FIG. 2 Numb and Prospero localization in dividing SOP cells. Embryos were double-stained with antibodies against Prospero (a–f, red) and Numb (g–l, green). a, g, Early prophase: Prospero can be detected throughout the cell, whereas Numb is localized to the membrane. b, h, Late prophase: Prospero colocalizes with Numb at the membrane. c, i, Metaphase: both proteins are asymmetrically localized. No differences in the time course of asymmetric localization were observed between Prospero and Numb in any cell. d, j, Early interphase: Numb and Prospero segregate into the same daughter cell. Numb remains membrane associated and Prospero translocates into the nucleus. Brackets indicate the position of the non-staining sister cell. e, k, *numb* mutants: Prospero is localized normally. f, l, *prospero* mutants: Numb is localized normally.



METHODS. Stage-11 embryos were double-stained with a rabbit anti-Numb antibody and a monoclonal Prospero antibody¹⁰. Counterstaining with the DNA dye Hoechst 33258 was used to determine the mitotic phase (not shown). Identical results on Prospero localization were obtained with a polyclonal rabbit anti-Prospero antibody⁶ (not shown). For mutant analysis the amorphic alleles *numb*¹ and *prospero*¹⁰¹³ were used^{5,6}.

mitotic spindle and accumulate in metaphase (Fig. 4b). The mitotic chromosomes are not aligned into the metaphase plate and the two centrosomes are found at varying random positions within the cell. Surprisingly, Numb still localizes in a crescent (Fig. 4d) that has the correct orientation relative to the surrounding tissues³. The position of the Numb crescent, however, is no longer correlated with either of the two centrosomes. A statistical analysis reveals that both establishment and maintenance of Numb localizations are not affected (Fig. 4e). No polymerized microtubules are detectable in immunofluorescence following a 5-min colcemid treatment (data not shown). However, the number of Numb and Prospero crescents is significantly increased as the treatment is prolonged to 20 min. Thus new crescents are formed in the presence of colcemid as more cells accumulate in metaphase. Similar results are obtained for the localization of Prospero (data not shown). These results suggest that the localization of Numb and Prospero is microtubule-independent, but recognizes positional information that is provided for the placement of the centrosome and the mitotic spindle.

To determine the actin dependence of Numb and Prospero localization, we treated *Drosophila* embryos with cytochalasin D (Fig. 4f–k). This treatment perturbs the cortical actin cytoskeleton (Fig. 4g) and blocks actin-dependent processes such as cytokinesis. However, both Numb and Prospero are still localized (Fig. 4h, j). Inhibition of cytokinesis leads to the formation of cells with two nuclei. Numb and Prospero are still asymmetrically localized in these cells during telophase (Fig. 4i, and data not shown). After mitosis, Numb redistributes evenly along the membrane, whereas Prospero enters both nuclei (Fig. 4k).

Our experiments suggest the existence of a novel mechanism for asymmetric protein segregation during mitosis, which is used by at least two proteins, Numb and Prospero. It functions independently of centrosome positioning and spindle orientation, but recognizes the same positional information. We propose the existence of an organizer of asymmetric cell division which governs several independent aspects of asymmetry during mitosis and provides positional information for both the orientation of the mitotic spindle and asymmetric Numb and Prospero localization. Coordination between asymmetric protein segrega-

FIG. 3 Numb forms a double crescent in *pebble* mutant embryos. a–d, The first 13 rounds of mitosis during *Drosophila* embryogenesis occur in a syncytium, and the first complete cell division during development is mitosis 14. In *pebble* mutants, mitosis 14 occurs without cytokinesis, giving rise to daughter cells with two nuclei and two centrosomes^{11,12}. These centrosomes duplicate normally during S-phase and in mitosis 15, cells have four centrosomes. a, Numb localization during mitosis 15 in a control embryo. DNA (red), the centrosome (green) and Numb (green) are shown. b–d, In *pebble* mutant embryos of the same age, dividing neuroblasts have four centrosomes and an increased amount of DNA. b, In 51% of these cells, the centrosomes form two pairs on opposite sides of the cell and Numb localizes into a tight crescent overlying one of these pairs. c, In 39% of these cells, the centrosomes are located individually and Numb forms a crescent that is centred between two of the four centrosomes. d, In 10% of these cells, the centrosomes are located further away from one another and Numb localizes into two crescents overlying two adjacent centrosomes. Because one of these two centrosomes is located in a different focal plane, confocal scans from two different planes were merged and the actual distance between these centrosomes is larger than in this image. METHODS. Embryos from *pebble*⁷⁰ and *pebble*¹¹⁰ mutant stocks^{11,12} were collected at the time of mitosis 15 (4.5–5.5 h at 25 °C) and stained as described for Fig. 1. No differences between these two amorphic *pebble* alleles could be detected. One quarter of the embryos were classified as homozygous mutant based on their abnormal DNA staining pattern. For statistical analysis, 118 neuroblasts from *pebble*⁷⁰ mutant embryos were counted. Only neuroblasts with four clearly visible centrosomes were analysed.

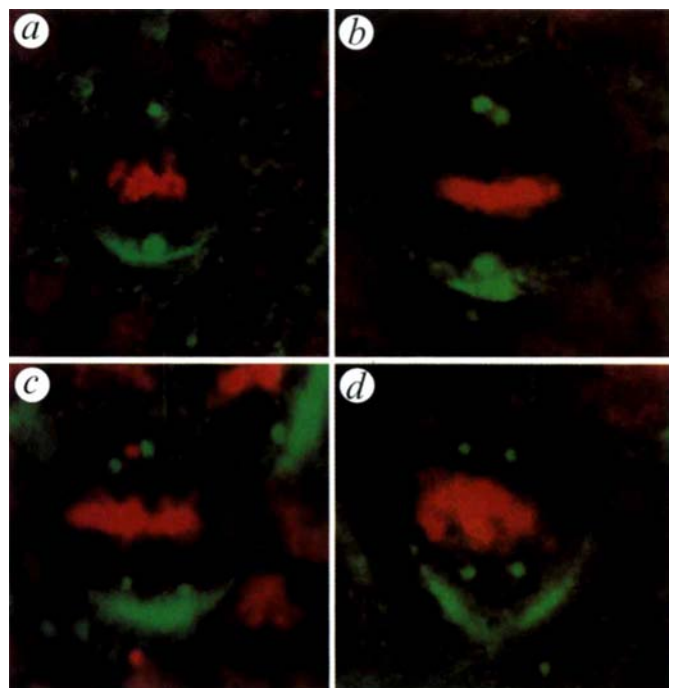


FIG. 4 Numb localization is unaffected by colcemid and cytochalasin treatment. *a–e*, Colcemid treatment. *a*, In a neuroblast of a control embryo the mitotic spindle (β -tubulin in green) aligns the chromosomes (red) in the metaphase plate. *b*, After colcemid treatment, no polymerized microtubules are visible (β -tubulin in green) and the chromosomes (red) are not aligned in the metaphase plate. *c*, In control embryos Numb localizes into a crescent overlying one of the two centrosomes (Numb and centrosomes in green, DNA in red). *d*, After colcemid treatment, the centrosomes are mislocalized but Numb localization is not impaired. *e*, Statistical analysis (mean $\pm$ standard deviation of 10 embryos). After 5 min of colcemid treatment, no polymerized microtubules are visible and cells accumulate in metaphase (data not shown). However, new Numb crescents can still form. After 20 min of colcemid treatment their number increases from 22 to 48. *f–k*, Cytochalasin D treatment. *f*, *g*, Actin cytoskeleton of a control embryo (*f*) and a cytochalasin D treated embryo (*g*) stained with rhodamine-phalloidin. The arrowhead shows a dividing neuroblast. *h*, Numb (green) is still localized after cytochalasin D treatment (DNA in red). However, in 22% of the cells, the position of the Numb crescent with respect to the surrounding tissues is not correct. *i*, Cytokinesis is inhibited and telophase cells with two nuclei (red) and a Numb crescent (green) are formed. *j*, *k*, After cytochalasin D treatment, Prospero (green, DNA in red) is still localized (*j*) and enter both nuclei (*k*) after mitosis.

METHODS. *a–d*, Stage-10 embryos were treated for 20 min with $20 \mu\text{g ml}^{-1}$ colcemid (essentially as described⁹) and stained for β -tubulin⁶ or for Numb and the centrosome (Fig. 1). *e*, Embryos were stained with Numb antibodies after a 5- or 20-min colcemid treatment. The number of crescents was counted in 10 laterally viewed embryos. *f–k*, Stage-10 embryos were treated for 30 min with $1 \mu\text{g ml}^{-1}$ cytochalasin D (as described for colcemid) and stained with rhodamine-phalloidin²⁷ or with Prospero⁶ or Numb³ antibodies and propidium iodide²⁶. No differences were detected when the cytochalasin D concentration was increased to $20 \mu\text{g ml}^{-1}$.

tion and spindle localization has been suggested before in *Caenorhabditis elegans*. During the first cell division of a *C. elegans* zygote, the P-granules segregate into one of the two daughter cells¹⁶. P-granule localization and spindle positioning are independent, but respond to a common cellular polarity¹⁷. The *par* genes are required for this polarity, but do not appear to function in later asymmetric cell divisions^{18–20}. In contrast to Numb localization, P-granule localization is sensitive to cytochalasin D treatment¹⁷. Furthermore, the actin cytoskeleton is highly polarized in the *C. elegans* zygote²¹, whereas no signs of asymmetric actin distribution are visible in dividing *Drosophila* neuronal cells²² (arrowhead in Fig. 4*f*). Thus different mechanisms probably underlie P-granule localization and the localization of Numb and Prospero.

The insensitivity of Numb localization to treatment with colcemid and cytochalasin constrains the possible cellular mechanisms. It is possible that during mitosis Numb acquires affinity for an already asymmetrically distributed receptor. Alternatively, binding of Numb molecules to each other could lead to asymmetric localization by a capping mechanism. What positions the organizer of asymmetric cell division? All the different cell types that localize Numb during mitosis originate from pola-

rized epithelial cells. Epithelial polarity could be inherited and translated into the observed asymmetry during cell division. In *Saccharomyces cerevisiae*, a GTPase cascade interprets a molecular mark that is left at the previous site of cytokinesis and determines the position of the new mitotic spindle²³. Whether homologous proteins are involved in controlling asymmetry during cell division in *Drosophila* remains to be determined. □

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Asymmetric segregation of the homeodomain protein Prospero during *Drosophila* development

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ASYMMETRIC divisions that produce two distinct cells play fundamental roles in generating different cell types during development^{1,2}. In the *Drosophila* central nervous system, neural stem cells called neuroblasts divide unequally into another neuroblast and a ganglion mother cell which is subsequently cleaved into neurons. Correct gene expression of ganglion mother cells requires the transcription factor Prospero^{3–5}. Here we demonstrate the

asymmetric segregation of Prospero on neuroblast division. Prospero synthesized in neuroblasts is retained in the cytoplasm and at mitosis is exclusively partitioned to ganglion mother cells, in which it is translocated to the nucleus. Differential segregation of Prospero was also found in the endoderm. We have identified a region in Prospero that is responsible for this event. The region shares a common motif with Numb⁶, which also shows unequal segregation⁷. We propose that asymmetric segregation of transcription factors is an intrinsic mechanism for establishing asymmetry in gene expression between sibling cells.

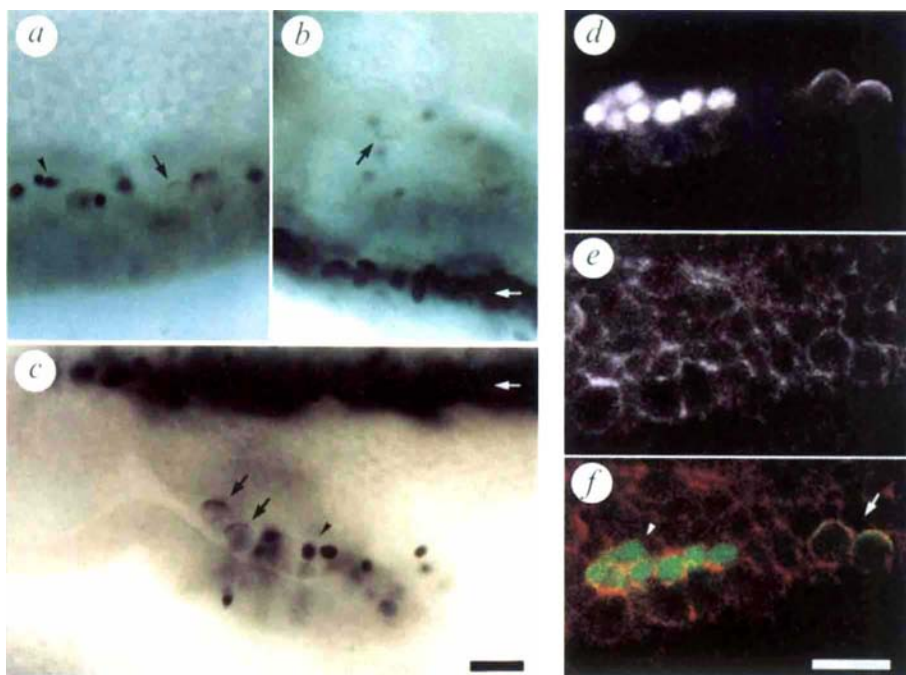
Although the *prospero* gene (*pros*) is transcribed both in neuroblasts and in ganglion mother cells (GMC)^{3–5}, its translation product has been observed only in the nuclei of GMCs^{4,5}. Careful examination of Prospero expression using a monoclonal antibody against it⁸ revealed that it was translated in neuroblasts but not localized to their nuclei (Fig. 1a). Prospero alters its subcellular localization in a cell-cycle-dependent manner (Fig. 2). In interphase, it is faintly detected in the cytoplasm (Figs 1d, 2a). When a neuroblast enters into the mitotic phase, it localizes cortically close to the cell membrane, and accumulates on its basal side (where a GMC will bud) in a crescent pattern (Fig. 2b) overlapping the cortical actin network (Fig. 1d–f). During anaphase when a bud emerges, Prospero moves into the bud (Fig. 2c, d) completely until telophase (Fig. 2e). Thus Prospero is segregated solely to a GMC from a parental neuroblast. Prospero persists in the cortical position of a GMC shortly after division until its translocation to the nucleus (Fig. 1a, f).

The asymmetric segregation of Prospero is not specific to the nervous system of ectodermal origin but also occurs in the embryonic endoderm (Fig. 1b, c). Large non-neural cells in the lumen of the midgut primordium⁹ express Prospero in the crescent pattern. During asymmetric divisions of those cells, Prospero is transported specifically to the small daughter cells where it localizes to their nuclei (Fig. 1c).

Several factors localize asymmetrically in *Drosophila* oocytes and early embryos^{10–13}, or between sibling cells in the *Caenorhabditis elegans* embryos¹⁴; localization of those factors in a cell or

FIG. 1 Asymmetric segregation of Prospero to daughter cells of stem cell division in the embryonic central nervous system and the endoderm. **a**, Lateral view of a *Drosophila* wild-type embryo at stage 10 (ref. 25). Anti-Prospero monoclonal antibody, MR1A⁸, stains the nuclei of GMCs (arrowhead) and the cytoplasm of a neuroblast in a crescent pattern (arrow). **b**, Anterior midgut primordium of a stage-11 embryo showing that a large stem cell is exporting Prospero into a newly forming small cell (arrow). **c**, Posterior midgut primordium of a stage-11 embryo. Large stem cells (arrows) in the lumen begin to express Prospero in a crescent pattern at mid-stage-11. Those cells divide asymmetrically to produce small cells to which Prospero is segregated, resulting in its nuclear localization (arrowhead). Prospero-positive small cells are non-neural but presumably adult midgut precursors²⁶. Anterior, left; dorsal, top (a–c). White arrows in **b** and **c** indicate Prospero expression in the central nervous system. **d–f**, Confocal images of a stage-11 embryo showing colocalization of Prospero with the cortical actin network in neuroblasts (arrow), in contrast to its nuclear localization in GMCs (arrowhead). Prospero (**d**, and green in **f**), and cortical F-actin (**e**, and red in **f**). Scale bar, 10 μ m.

METHODS. Fixed embryos were stained with a monoclonal anti-Prospero antibody, MR1A, which was raised against its homeodomain⁸ (provided by E. Spana and C. Doe), followed by biotinylated anti-mouse IgG and ABC elite kit (Vectastain), and viewed in an Axiophoto microscope



(Zeiss). For confocal image analysis, avidin-Cy 3 (Molecular Probes) was used to label MR1A, and fluorescein-phalloidin to label F-actin. Stained embryos were observed with a Sarastro-2000 confocal microscope (Molecular Dynamics).

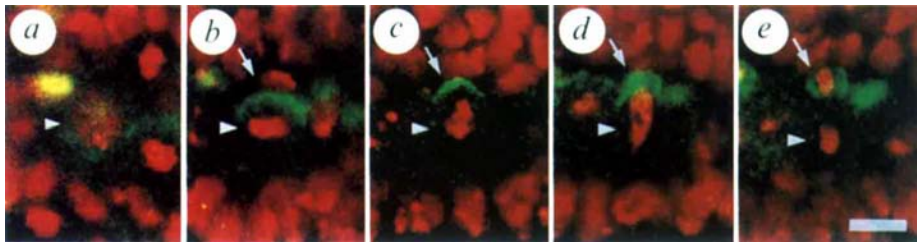
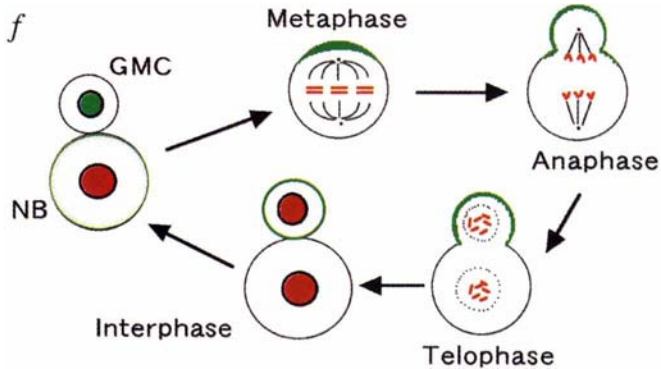


FIG. 2 Cell-cycle dependence of subcellular localization of Prospero (green) in neuroblasts: a, interphase; b, metaphase; c, early anaphase; d, late anaphase; e, telophase. Confocal microscopy visualizes segregation of Prospero to a GMC from a parental neuroblast (arrows), as well as the morphology of chromosomes (red, arrowheads). Scale bar, 5 μ m. f, Schematic representation of subcellular localization of Prospero during asymmetric division of a neuroblast. Localization of Prospero is shown in green; chromosomes are in red. NB, neuroblast. GMC, ganglion mother cell.

METHODS. Embryos of stages 10–11 were stained with MR1A as described in Fig. 1 legend. TOTO-3 (Molecular Probes) was used to stain chromosomes and recorded in the Cy-5 channel.



between sister cells is regulated by signals in their messenger RNAs, not in protein sequences (reviewed in refs 15, 16). To test this as a possibility for Prospero, we expressed two chimaeric genes between a full-length *prospero* complementary DNA⁵ and the *Escherichia coli lacZ* gene encoding β -galactosidase in flies; one is the *prospero* cDNA connected in-frame to the *lacZ* gene with a nuclear localization signal (NLS) (Fig. 3a, ProsLacZ),

and the other, the *prospero* cDNA out of frame with the preceding *lacZ* gene so that only the *lacZ* part is translated (Fig. 3a, LacZUTRpros). Anti- β -galactosidase antibody detected the ProsLacZ protein, showing the subcellular localization to be essentially identical to Prospero in neuroblasts before its differential segregation to GMCs (Fig. 4a, b, d). In contrast, the translation product from the out-of-frame construct is uniformly

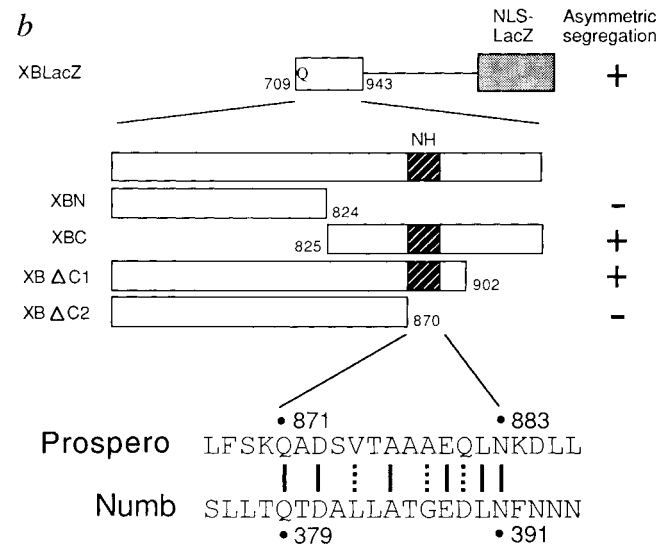
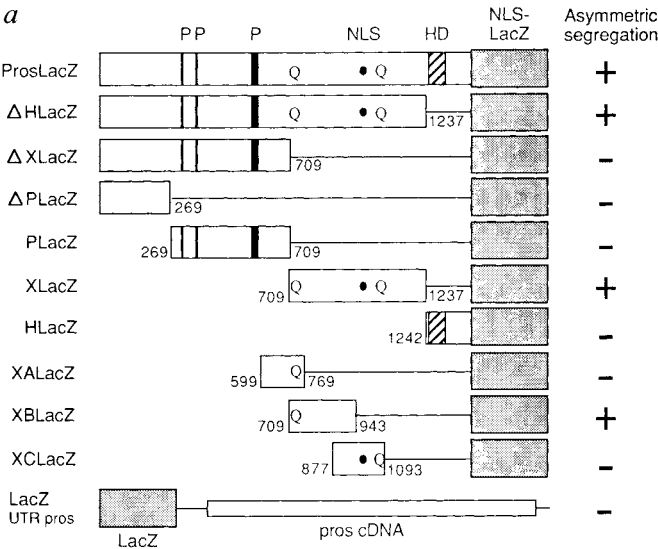
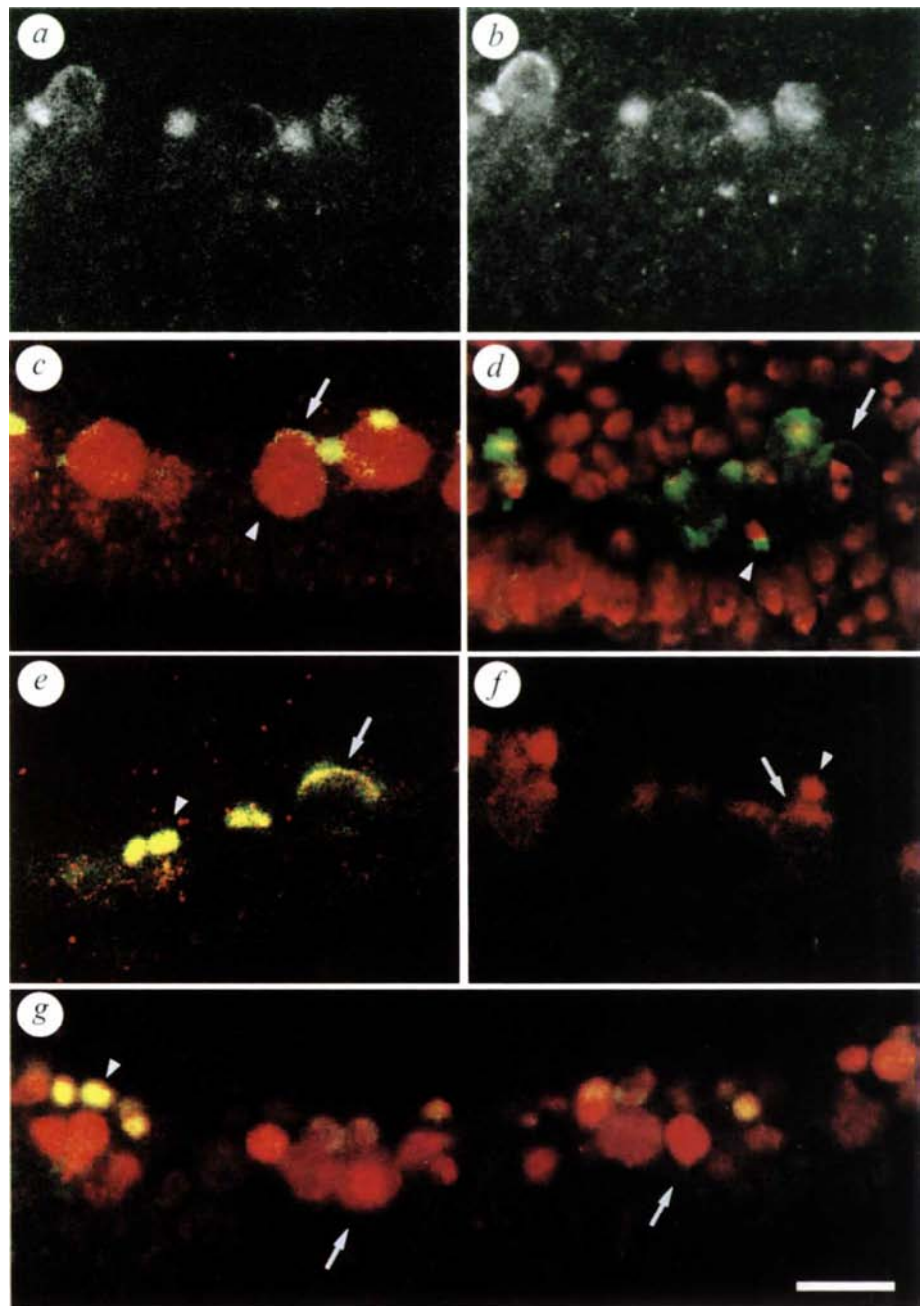


FIG. 3 a, Determination of a domain responsible for asymmetric segregation of Prospero. Chimaeric genes were made between a series of deleted full-length *prospero* cDNAs and the NLS-*lacZ* gene. Each chimaeric gene encodes a portion of Prospero in-frame with NLS- β -galactosidase. Plus sign indicates fusion proteins showing an identical localization to endogenous Prospero; minus sign indicates that fusion proteins localize to the nuclei in neuroblasts, then distribute evenly between two cells after division as NLS- β -galactosidase (Fig. 4). The region corresponding to amino acids 709–943 (XBLacZ) is sufficient for asymmetric segregation of its NLS-*lacZ* fusion proteins in *prospero* deficient embryos. b, Analysis of the XB region. The C-terminal half (XBC; amino acids 825–943) of the XB region confers asymmetric segregation on NLS- β -galactosidase. Deletion of the C-terminal 41 amino acids from the XB region (XBAC1) does not affect asymmetric segregation of its

fusion protein, but deletion of a further 32 amino acids deprives XB of its activity (XBAC2). This essential region (amino acids 871–902) contains a sequence similar to the 13 amino acids of Numb (amino acids 379–391). Vertical bars indicate identity; dots, conservative matches; HD, homeodomain; NLS, nuclear localization signal; P, PEST sequence; Q, opa repeat; NH, homology with Numb.

METHODS. The NLS-*lacZ* gene was derived from pENL containing a nuclear localization signal of SV40 large-T antigen in front of the *lacZ* gene (gift from M. Tanaka). Chimaeric genes were inserted downstream of the 5-kb *pros* EcoRI fragment in the pCaSpeR vector²⁷. This fragment activates transcription of the chimaeric genes essentially in the same way as the *pros* gene. Plasmids were introduced into *white* flies by P-element-mediated germline transformation and independent lines were established for each transformant.

FIG 4. Confocal images showing localization of the fusion proteins (see Fig. 3) in the ventral nervous system. *a, b*, Embryo expressing the fusion protein translated from the ProsLacZ construct was doubly stained with MR1A (*a*) and anti- β -galactosidase antibody (*b*), showing that the localization of the fusion protein is basically identical to Prospero. MR1A detects endogenous Prospero as well as the fusion protein in this embryo. *c*, Embryo carrying the LacZUTRpros construct in which the full-length *prospero* mRNA sequence is out of frame (see Fig. 3a). In contrast to endogenous Prospero (green, arrow), β -galactosidase from the construct (red, arrowhead) fills the cytoplasm of neuroblasts. *d*, The ProsLacZ protein (green) shows a cell-cycle-dependent localization similar to Prospero (Fig. 2). TOTO-3 (red) stains chromosomes. Arrow indicates an anaphase neuroblast. Staining in the apical cytoplasm of a telophase neuroblast (arrowhead) suggests that the protein might be retained by centrosomes. *e*, XBLacZ protein (red) colocalizes with endogenous Prospero (green) in a wild-type background, resulting in yellow staining of the GMC nuclei (arrowhead) and a crescent in the neuroblast (arrow). *f*, XBLacZ protein (red) in a *prospero*-deficient embryo also shows crescent localization in a neuroblast (arrow) and subsequent nuclear translocation in a GMC (arrowhead) in the absence of the endogenous Prospero (green). *g*, Δ XBLacZ protein (red) distributes equally between the nuclei of both GMCs (arrowhead) and neuroblasts (arrows) in interphase, in contrast to endogenous Prospero (green). Colocalization is indicated by yellow coloration. Scale bar, 10 μ m. METHODS. Staining with anti- β -galactosidase antibody was visualized using avidin-Cy 3 (Molecular Probes); MR1A, Cy 5. The *prospero*^{C7} mutation, a null allele of the *prospero* locus⁵, was introduced into a strain bearing the XBLacZ construct (*f*).



distributed in the cytoplasm of neuroblasts and evenly segregated to daughter cells upon cytokinesis (Fig. 4c). These results show that asymmetric segregation of the ProsLacZ fusion protein requires translation of *prospero* mRNA.

We mapped the region responsible for asymmetric Prospero segregation along its sequence by making a set of chimaeric genes (Fig. 3a), in which a part of *prospero* is fused in-frame with the NLS-lacZ reporter gene. A region encompassing amino acids 709–943 of the *prospero* polypeptide was identified as sufficient for differential segregation (XBLacZ in Figs 3a, 4e, f). This region is capable of localizing NLS-LacZ in *prospero*-deficient embryos (Fig. 4f), excluding the possible contribution from endogenous Prospero to the asymmetric localization of the fusion protein. Fusion proteins without this region are evenly distributed between daughter cells (Fig. 4g). Further analysis indicates that the carboxy-terminal half (amino acids 825–943) of this region is active in differential segregation (Fig. 3b). In addition, C-terminal deletion of the XB region reveals that the

domain between amino acids 871 and 902 is essential (Fig. 3b). This domain contains a short motif homologous to Numb⁶ (Fig. 3b) which distributes asymmetrically in neural precursors in a similar manner to Prospero but remains in the cell cortex⁷. The presence of a motif in Numb similar to the Prospero 'asymmetry' region indicates that a common mechanism may be responsible for asymmetric segregation. The region identified here should include domains necessary for trapping Prospero in the cytoplasm, in spite of its nuclear localization signals^{17,20}, and for subsequent exportation to the bud of division^{7,21,22}.

Differential translation, transcription or subcellular localizations of mRNA are all mechanisms for differential expression of transcription factors in cells (see refs 15, 16 for review). As Prospero binds to specific DNA sequences *in vitro* (S. Goto and F.M., unpublished results) like other homeodomain proteins acting as transcriptional regulators²³, this protein is, to our knowledge, the first example of a DNA-binding factor that segregates asymmetrically to one daughter cell during division. Our

results suggest an alternative mechanism that might enable sister cells to regulate gene expression differentially immediately after division²⁴, thereby generating asymmetry in proliferating cells such as neuroblasts. Our observation in endodermal cells suggests that this type of differential gene regulation occurs widely during development. The 'asymmetry' domain identified here will help our understanding of the basic mechanism that generates asymmetry in mitosis^{1,2}. □

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A role for CD95 ligand in preventing graft rejection

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TESTIS is a remarkable immune-privileged site, long known for its ability to support allogeneic and xenogeneic tissue transplants^{1–4}. Here we have investigated the molecular basis for testis immune privilege. Testis grafts derived from mice that can express functional CD95 (Fas or Apo-1) ligand^{5–8} survived indefinitely when transplanted under the kidney capsule of allogeneic animals, whereas testis grafts derived from mutant *gld* mice, which express non-functional ligand^{8,9}, were rejected. Further analysis of testis showed that CD95 ligand messenger RNA is constitutively expressed by testicular Sertoli cells, and that Sertoli cells from normal mice, but not *gld* mice, were accepted when transplanted into allogeneic recipients. CD95 ligand expression in the testis

probably acts by inducing apoptotic cell death of CD95-expressing, recipient T cells activated in response to graft antigens. These findings indicate that CD95 ligand could be used to create immune-privileged tissue for a variety of transplant uses.

CD95 and its ligand (CD95L) are cell-surface molecules that interact in the regulation of immune responses^{5,10–12}. Resting T cells normally express low levels of CD95. However, within hours of engagement of their antigen receptors, CD95 expression increases significantly. Several days after initial activation, CD95 becomes 'functional' and the T cells undergo apoptosis in response to crosslinking with anti-CD95 antibodies^{13,14}. The ability to express functional CD95 is lacking in *lpr* mice, which cannot eliminate activated lymphocytes and eventually develop autoimmune disease^{15,16}.

In vivo, crosslinking of CD95 is mediated by CD95L either as transmembrane or soluble secreted molecules^{5,8}. *Gld* mice lack expression of functional CD95L and exhibit a disease identical to that of *lpr* mice. CD95L protein is synthesized and transiently expressed by CD8⁺ cytotoxic T lymphocytes (CTL) and by CD4⁺ T helper 1 cells (Th1) when their antigen receptors are engaged^{6,16–20}. CD95L expression occurs even when CD95 is being upregulated on the same and other T cells during an immune response. Thus a T cell might have only three or four days to perform its effector function before being targeted for destruction by suicide or fratricide/soricide^{21–25}. CD95-mediated, apoptotic cell death limits immune responses in general and may provide a safeguard against emergence of autoreactive lymphocytes^{15,20,21}.

In addition to activated CTL and Th1, CD95L mRNA has been observed in rodent, but not human, testis^{5,7}. We have previously reported that rat testis, when placed in the abdominal cavity, is a highly effective immune-privileged site into which both allografts and xenografts can be successfully transplanted^{3,26–29}. We hypothesized that expression of CD95L in testis could account for its immune-privileged status, and used a transplant model to test this. When even weakly immunogenic islet or thyroid tissue from B6 donors was transplanted under the kidney capsule of BALB/c recipients, it was destroyed within two weeks (data not shown). This was expected because BALB/c and B6 mice are genetically incompatible at the major histocompatibility complex (MHC) as well as at multiple minor loci. When similar experiments were performed with testis tissue (Fig. 1a), we found that B6 testis grafts were maintained and that their appearance after 21 days of transplantation in BALB/c recipients was indistinguishable from syngeneic grafts (compare Figs 1b and 1c). There was no evidence of lymphocytic infiltration even after examination at higher magnification (Fig. 1d). These experiments demonstrated that allogeneic testicular tissue, unlike tissue from virtually any other source including pancreatic islet cells, thyroid and skin, was protected from T-cell-dependent allograft destruction.

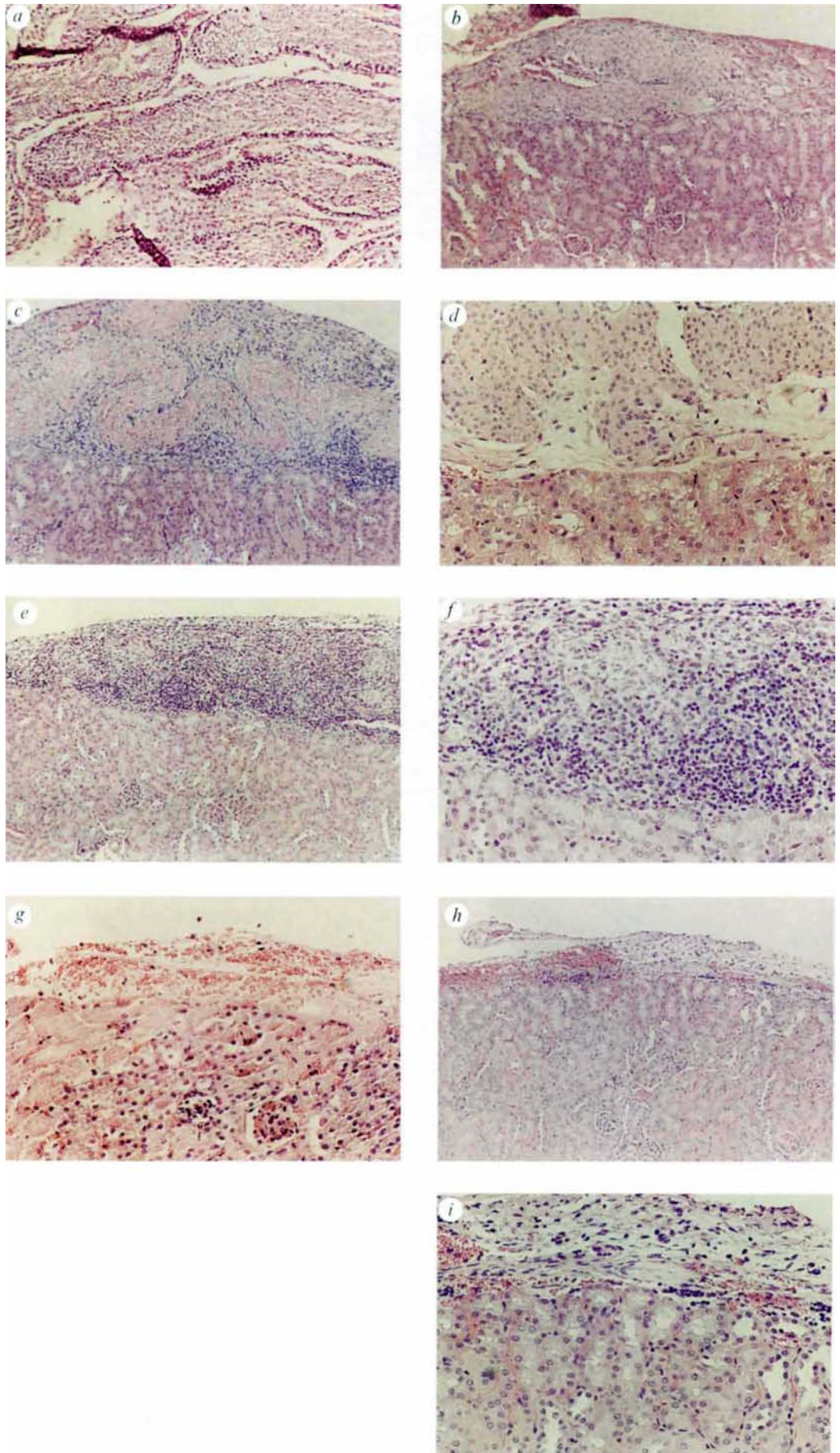
Previous studies had indicated that the ability of testis to protect against allograft rejection was provided by Sertoli cells^{27–29}. Sertoli cells isolated from B6 mouse testis by enzymatic digestion were transplanted under the kidney capsule of BALB/c recipients. B6 Sertoli cells showed no evidence of rejection when examined 21 days after transplantation (Fig. 1e, f).

In contrast to the results obtained with B6 testis, we found that when testis tissue derived from B6Snn.C3H-*gld* (B6-*gld*) mice was transplanted under the kidney capsule of BALB/c recipients it was promptly rejected (Fig. 1g). Sertoli cell grafts derived from B6-*gld* donors were also rejected (Fig. 1i, h). Essentially no tissue could be found at day 7. Similar experiments using B6.MRL-*lpr* (B6-*lpr*) mice, which express wild-type CD95L but lack functional expression of CD95, indicated that B6-*lpr* testis grafts, like those of B6, were accepted in BALB/c recipients.

To demonstrate formally that Sertoli cells constitutively expressed CD95L transcripts, reverse transcription-polymerase chain reaction (RT-PCR) analysis was performed (Fig. 2). As

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FIG. 1 Histology of BALB/c, B6 and B6-*gld* testis or Sertoli cells transplanted under the kidney capsule of BALB/c mice. **a**, Photomicrograph (magnification $\times 45$) of testis tissue before transplantation. **b**, BALB/c testis graft after 21 days ($\times 45$). Although germinal tissue has deteriorated owing to its placement at 37 °C, the histological appearance of the transplanted testicular tissue is readily recognizable (compare **a** and **b**). **c**, B6 testis graft after 21 days ($\times 45$), and **d**, after 21 days ($\times 113$). Note the lack of lymphocytic infiltration in **c** and **d**. **e**, B6 Sertoli cell graft after 21 days ($\times 45$) and **f**, after 21 days ($\times 113$). Graft acceptance is clearly recognized in **e** and **f**. **g**, B6-*gld* testis graft after 2 days ($\times 45$). **h**, B6-*gld* Sertoli cell graft after 7 days ($\times 45$), and **i**, after 7 days ($\times 113$). Extensive infiltration of lymphocytes in the B6-*gld* grafts is observed after 2 days, at which time the architecture of the testis tissue is severely disrupted. The renal tissue also shows obvious lymphocytic infiltration adjacent to the graft. METHODS. In the nine colour photographs of testis tissue or Sertoli cells isolated from testis tissue, either B6, B6-*gld* or BALB/c mice aged 8–12 weeks were used as donors. At various time points after the tissue was transplanted (days 2 and 7 for B6-*gld*, and days 2, 7, 21 and 28 for B6 and BALB/c tissue), the BALB/c recipient was killed and the kidney containing the graft was removed, fixed in formalin and processed by routine histological techniques. In total, 34 B6 testis grafts, 34 B6-*gld* grafts, 12 B6 Sertoli cells grafts, and 12 B6-*gld* Sertoli cell grafts were examined. The data shown are representative of at least 5 animals per time point.



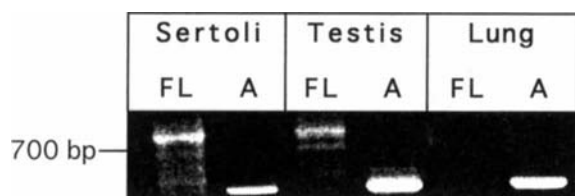


FIG. 2 CD95L mRNA is expressed in Sertoli cells. RT-PCR analysis was performed on RNA isolated from mouse Sertoli cells, testis and lung. CD95L (FastLane: FL) was expressed in testis and isolated Sertoli cells from testis, but not in lung. Actin (A) was used to normalize for RT-PCR analysis of the isolated RNA.

METHODS. Total RNA was isolated from C57Bl/6 testis, thymus, liver, lung and Sertoli cells using TRIzol reagent (Gibco/BRL), according to the manufacturer's instructions. First strand complementary DNA synthesis was performed using 5 µg of total RNA in the presence of Superscript II reverse transcriptase and oligo (dT)₁₂₋₁₈ primer. Reaction mixtures for PCR contained cDNA template, 1 µM each of sense and antisense primers, and a combination of 2.5 U Taq DNA polymerase (Perkin-

shown previously by northern analysis⁷, CD95L expression was observed in testis but not in lung. Relevant to the results presented above, CD95L transcripts were detected in freshly isolated Sertoli cells as well as in Sertoli cells cultured at 32 °C and 37 °C (data not shown) in consideration of the temperatures found in the scrotal and abdominal positions for transplantation. Our results indicate that at least part of the reported expression of CD95 ligand in rodent testis^{5,7} can be attributed to Sertoli cells.

In summary, our findings suggest that expression of functional CD95L by Sertoli cells accounts for the immune-privileged nature of testis. The link between the failure of B6-*gld* Sertoli cells to protect against allograft immunity (Fig. 1) and the inability of B6-*gld* T cells to mediate *in vitro* cytotoxicity against syngeneic activated T-cell targets¹⁰ is due to the loss of critical interactions between CD95 and its ligand. Consistent with this model, preliminary studies have shown that BALB/c testis was rejected by B6-*lpr* mice (data not shown).

CD95 ligand-mediated immunosuppression would be expected to primarily target activated effector cells rather than the activation steps that produce them. The most commonly used immunosuppressive agents, such as cyclosporin A, target T-cell lymphokine production but need not block effector function. It is not surprising that these agents might be less effective against previously activated T cells³⁰. By targeting only activated T lymphocytes, grafted cell-associated CD95L may provide a highly specific form of immunosuppression for ameliorating T-cell-dependent graft rejection. □

Elmer/Cetus) and Vent (exo⁺) DNA polymerase (NEB) in a ratio of 40:1 to ensure PCR fidelity. The CD95L sense primer (CGGGATCCATGCAGCA-GCCCTTCAATTAC) flanked the ATG initiation codon and included a *Bam*H1 recognition site at the 5' end for cloning; the antisense primer (CGGAATTCCTCTTAGAGCTTATATAAGCC) flanked the TAA termination codon including an *Eco*R1 recognition site. The upstream nested primer sequence (CGGGATCCATGCAGCTCTCCACCTACAG), with an added *Bam*H1 recognition site, initiates at nucleotide 426 following the transmembrane region of the CD95L gene product. Amplification consisted of 30 cycles of denaturation (72 °C for 30 s), annealing (55 °C for 30 s) and extension (72 °C for 1 min). The PCR products were resolved on 0.8% agarose gels. Positive controls included full-length CD95L cDNA prepared from RNA isolated from mouse CTL stimulated with PMA and ionomycin. Verification of CD95L PCR product specificity was accomplished by nested PCR amplification of full-length reaction products and by restriction enzyme analysis. Controls for RNA integrity and tissue RNA normalization consisted of PCR amplification of F-Actin 3' untranslated region sequence, using the 5' primer (GATGCATTGTTACAGGAAGT) and 3' primer (TCATACATCTCAAGTTGGGG), producing a 240-bp fragment corresponding to nucleotides 3260–3500 of the gene, from oligo (dT)-primed cDNA.

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Protection against mycoplasma infection using expression-library immunization

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As is evident from the human immunodeficiency virus epidemic, there is no systematic method for producing a vaccine. Genetic immunization¹ is a new approach to vaccine production that has many of the advantages of live/attenuated pathogens but no risk of infection. It involves introducing DNA encoding a pathogen protein into host cells and has shown promise in several disease models^{2–13}. Here we describe a new method for vaccine development, expression-library immunization, which makes use of the technique of genetic immunization and the fact that all the antigens of a pathogen are encoded in its DNA. An expression library of pathogen DNA is used to immunize a host thereby producing the effects of antigen presentation of a live vaccine without the risk. We show that even partial expression libraries made from the DNA of *Mycoplasma pulmonis*, a natural pathogen in rodents, provide protection against challenge from the pathogen. Expression library immunization may prove to be a general method for vaccination against any pathogen.

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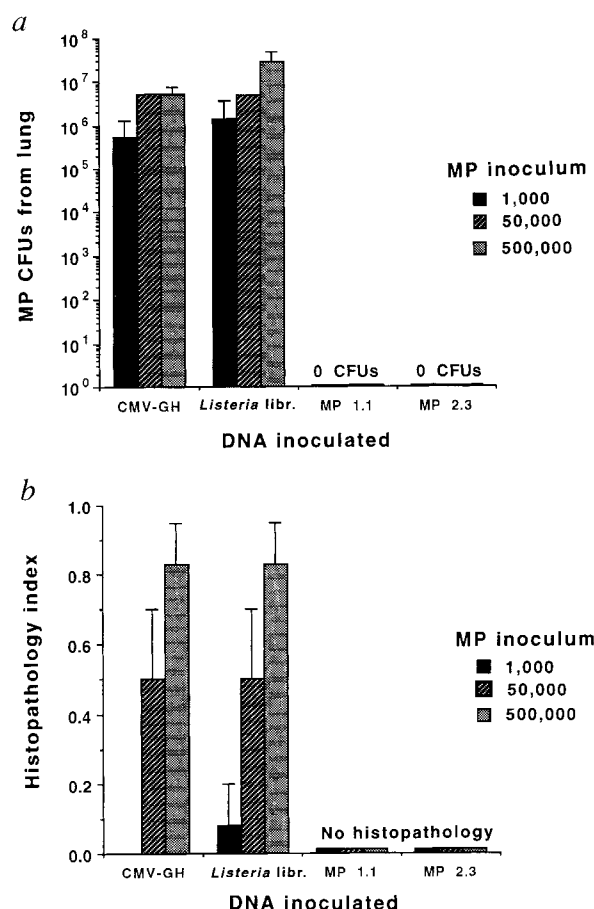


FIG. 1 MP titres and histopathology from ELI-immunized, MP-challenged mice. Mice were immunized with the indicated DNA and challenged with the indicated number of MP. CMV-GH expresses hGH. *Listeria* library is a hGH ELI library constructed using *Listeria monocytogenes* DNA. MP1.1 and MP2.3 are 3,000 transformant sibs of the mycoplasma library in which MP DNA was fused to coding frames 1 and 2, respectively, of hGH. a, MP titres. MP CFUs from lung represents the number of MP colony-forming units from each group of mice. b, Histopathology: the histopathological lesions induced by MP infection were scored from lung sections of mice by a histopathology index. An index of 1.0 represents the maximum number of lesions observed in infected mice. An index of zero indicates normal morphology. Each bar represents the mean from 2 to 4 mice. Error bars represent the standard deviation for each group.

METHODS. CMV-GH-F1 and -F3 and CMV-GH-F2 were constructed by inserting GATCTTGGATCTAAGTAAGTA and GATCGGATCTAAGTAAGTA, respectively, at the endogenous *Bgl*III site in exon 5 of hGH and *Hind*III site of the vector CMV-GH, a derivative of CMV1 (ref. 27) containing hGH from CMV-GH-ori²⁸. Genomic DNA from MP or *Listeria* was digested with *Mbo*I to a median size of 0.5 kb and ligated into the *Bam*HI or *Bgl*III sites of CMV-GH-F1 and -F3 and CMV-GH-F2. TG1 bacteria were transformed and plasmid DNA was purified from pools of 3,000 transformants using Qiagen columns. 6-week-old female BALB/c mice were genetically immunized as described²⁸ on day 1 with 10 µg DNA and 5 µg on days 8, 21 and 52. Mice were challenged on day 64, and lung lavage and sectioning was done 14 days later as described²².

We tested expression-library immunization (ELI) against *Mycoplasma pulmonis* (MP) because it is a normal lung pathogen in rodents^{14,15}, has a relatively small genome (~1 × 10⁶ base pairs¹⁶), and has an unusual codon usage (for example, its tryptophan codon is a stop codon in eukaryotes^{17,18}). An ELI library was made by fusing digested *M. pulmonis* DNA onto the last exon of the gene encoding human growth hormone (hGH), such that hGH-MP antigens might be secreted. To capture all antigens, MP DNA was fused into three frames of hGH and nine

libraries of about 3,000 members (sibs) were prepared. Theoretically, a library expressing the entire genome of a pathogen could be used as a vaccine, but we found that ~1 ng DNA was required to produce an immune response by genetic immunization into the skin (data not shown and ref. 19). As 1–4 µg DNA is delivered per inoculation, libraries of 10³–10⁴ members were tested.

Two sib libraries, MP1.1 and MP2.3, were inoculated into separate groups of mice over 60 days with four inoculums totalling 25 µg DNA. This inoculum includes ~2 µg of MP DNA, representing ~1 × 10⁹ MP genomes or 10⁶-fold more than introduced in a normal MP infection. As negative controls, the hGH plasmid or an hGH *Listeria monocytogenes* ELI library was also inoculated. After sixty days, the immunized mice were challenged with MP and tested for infection two weeks later (Fig. 1a). Control mice had 10⁵–10⁷ mycoplasma in lung lavages even after the lowest (10³) challenge. Lung sections from these mice showed significant lesions (Figs 1b and 2). By contrast, mice inoculated with MP libraries had no culturable mycoplasma and no lung lesions (Figs 1 and 2). Protection by the MP libraries was pathogen-specific, because there was no protection by the *Listeria* library. This experiment was repeated with three inoculations over 30 days, followed by challenge with a more virulent isolate of the pathogen, and gave ~4 logs of protection as compared with control mice (results not shown). This indicates that library screening could be performed over shorter periods.

Figure 3 displays several important features of this technology. First, protective immunity conferred by ELI increases over time. Mice challenged with a more pathogenic isolate of MP had only partial protection after 60 days, but were fully protected after 100 days (Fig. 3 and data not shown). Second, a single immunization with an ELI library can confer lasting immunity.

TABLE 1. Immune responses induced by ELI libraries

	Anti-hGH antibodies*	Anti-MP antibodies†	MP-specific DTH (mm)‡	MP-specific MMI (%)§
Control	—	—	2.3 ± 0.4	0
MP 1.1	+	+	16.4 ± 0.4	71.8
MP 2.3	+	+	19.8 ± 0.3	73.3

Mice were immunized as described for Fig. 1. Sera for antibody tests were recovered 10 days after the second inoculation. Two mice from each group were tested for DTH and inhibition of macrophage migration (MMI) 12 days after the last immunization. Control refers to unimmunized mice.

*Antibody levels against hGH protein: —, designates no antibodies; +, levels detectable by enzyme-linked immunosorbent assay (ELISA) only at dilutions of 1:250.

†Antibody levels against whole mycoplasma antigens: —, no antibodies; +, levels detectable by ELISA only at dilutions of 1:50.

‡MP-specific delayed-type hypersensitivity. Delayed-type hypersensitivity was evaluated as described²² by injecting PBS buffer into the right rear footpad, and PBS containing 50 µg sonicated MP-cell protein into the left rear footpad. A dial-gauge calliper was used to measure the change in footpad thickness induced 24 h after injection. Three readings were measured and averaged. Measurements indicate the change in footpad thickness induced by injection of MP antigens in PBS minus that of PBS alone. Net footpad thickness (×100 mm) = ((mm post-MP injection – mm pre-MP injection) – mm post-PBS injection).

§MP-specific macrophage migration inhibition. Macrophage migration inhibition was evaluated as described²² by packing a glass capillary tube with 100 µl spleen-cell suspension (1 × 10⁶ cells per ml) from each mouse and placing it horizontally in a well of a 24-well plate immersed in RPMI medium in the absence or presence of 50 µg ml⁻¹ sonicated MP protein. After 24 h, the area of cell migration out of the tube was measured by digital imaging. Lower migration is indicative of release of macrophage migration inhibition factor (MIF) from T cells previously activated against MP antigens by immunization. Release of MIF in this assay results in reduced area of migrated cells from the capillary. Percent inhibition was calculated from the formula: (A – B) / A × 100, where A is the area of macrophage migration in medium and B is the area of macrophage migration in medium containing MP antigen.

FIG. 2 Lung-tissue sections from ELI-immunized, MP-challenged mice. Lungs from the mice used for Fig. 1 were fixed in formalin, sectioned, and stained with eosin and haematoxylin. *a* and *b*, Lung section from a mouse immunized with CMV-GH and challenged with 5×10^5 MP viewed at $1.5 \times$ and $10 \times$ magnification, respectively. The dark purple staining of mononuclear cell infiltration around bronchiole and blood vessels of the lungs (marked as L) indicates the lesions induced by massive infection with MP. The thymus is also evident and is marked as T. *c* and *d*, Lung section from a mouse immunized with library MP1.1 and challenged with 5×10^5 MP viewed at $1.5 \times$ and $10 \times$ magnification, respectively. No mononuclear cell infiltration is observed, indicating the absence of MP infection. The thymus is marked as T.

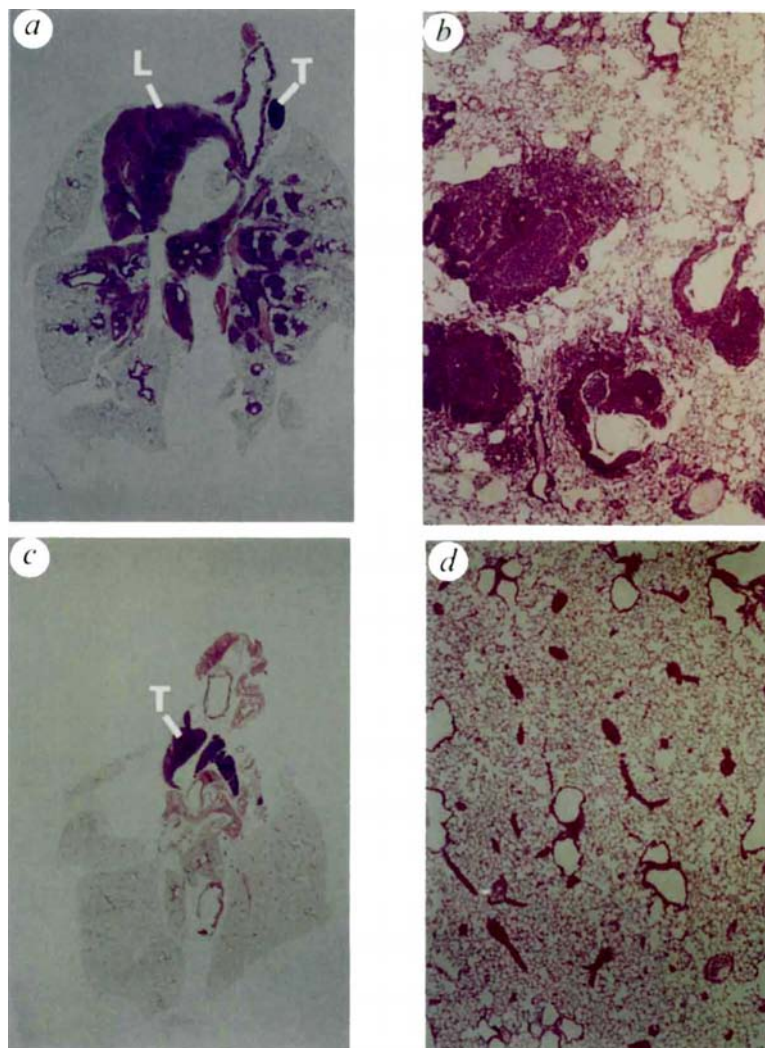
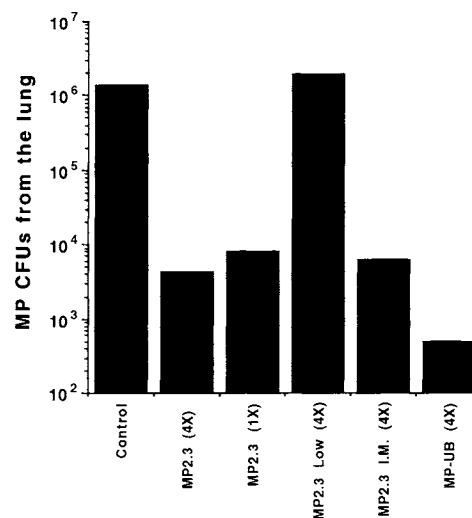


FIG. 3 Comparison of the route, dosage, length of protection and number of inoculations required to protect against a highly pathogenic isolate of MP. Mice were immunized over 60 days before challenge with a more virulent isolate of MP than that described in Fig. 1 legend. (1X), A single inoculation of MP2.3 60 days before challenge; (4X), 4 immunizations over 60 days, as for Fig. 1; MP2.3 Low, inoculation with $1 \mu\text{g}$ at each immunization (total, $4 \mu\text{g}$), compared to a normal total inoculum of $25 \mu\text{g}$; MP2.3 I.M., mice immunized by intramuscular injection $4 \times$ with $100 \mu\text{g}$ of MP2.3; MP-UB, mice immunized with a total of $25 \mu\text{g}$ of a library created by fusing MP DNA to mouse ubiquitin rather than to GH. Each bar represents the average for two mice. Although only a small number of mice were used in this screening experiment, the relative differences in MP titres between groups were substantial. The specific number of MP from the lung for each mouse were: control, 3.0×10^5 and 2.2×10^6 ; MP2.3 (4X), 7.7×10^3 and 3.3×10^3 ; MP2.3 (1X), 5.0×10^2 and 1.1×10^3 ; MP2.3 Low (4X), 4.9×10^5 and 3.4×10^6 ; MP2.3 I.M. (4X), 4.0×10^2 and 1.2×10^4 ; MP-UB (4X), 1.0×10^3 and 0. METHODS. Experiments were performed as for Fig. 1 except where indicated. The gene for mouse ubiquitin was amplified by PCR from BALB/c genomic DNA with two mutagenic oligonucleotides. One places a *Bam*HI 5' to the gene, supplies a Kozak start ATG, and knocks out the endogenous *Bgl*II site at amino acid 3 (CTCAGGATCCCACCATGCA-GATTTCGTGAAG). The other changes the final amino acid from Gly to Ala to inhibit cleavage of proteins fused to the carboxy terminus of ubiquitin²⁹ and adds a *Bgl*II site 3' to the coding sequence (GCTCAA-GATCTGCACCTCTCAGGCG). This PCR product was digested with *Bam*HI and *Bgl*II, then cloned into the *Bgl*II site of CMVB. Frame-insertion sites were added as described for the GH library in Fig. 1 legend.



A single inoculation of 10 µg MP2.3 60 days before challenge gave protection comparable to four inoculations totalling 25 µg over the same time (Fig. 3). Third, a threshold of antigen expression must be exceeded to produce protection. Four inoculations of only 1 µg gave no protection (Fig. 3). Finally, ELI libraries confer protective immunity when delivered in larger amounts by intramuscular injection, another method of genetic immunization. Injection of 100 µg of MP2.3 by this route four times over 60 days conferred a level of protection against the pathogenic strain similar to that achieved using 16-fold less DNA with the gene gun (Fig. 3).

Antibody and cellular immune responses induced by conventional vaccines have variable protective effects against MP in rodents^{15,20-24}. T cells from the library-immunized mice were primed against MP, as demonstrated by positive inhibition of macrophage migration and by strong delayed-type hypersensitivity (DTH) reactions (Table 1). Sera from library-immunized mice had low titres of antibodies against hGH and mycoplasma proteins. The low hGH antibodies suggest that some antigens fused to hGH may block normal secretion of hGH. Preliminary experiments indicate that ubiquitin-MP libraries give similar or better protection than hGH-MP libraries (Fig. 3), suggesting that a protective cellular immunity is elicited by ELI vaccines, because ubiquitin should drive antigens to the proteasome²⁵ for subsequent major histocompatibility complex class I presentation²⁶.

In summary, ELI can produce a non-infectious multipartite vaccine, even when little is known of the pathogen's biology. The primary limitation of genetic immunization—knowing which pathogen gene to use—is circumvented by ELI, which uses the immune system to screen candidate genes. MP1.1 and MP2.3 have $\sim 3 \times 10^3$ members, of which ~ 500 should be in-frame. The protection offered by both libraries indicates that there must be several different protective plasmids in each. Preliminary tests of two 69-member sibs derived from MP2.3 gave no protection, suggesting that the protective plasmid(s) in MP2.3 are located elsewhere in the library. We are currently testing the sibs from MP libraries to isolate these protective members. Once isolated, these genes could be used as genetic vaccines or to develop recombinant protein vaccines. ELI may be the fastest method to isolate protective genes from a pathogen, and, for pathogens that are difficult to grow or attenuate, it could provide the only avenue to an effective vaccine. Cancers or pathogens with larger genomes should be amenable to ELI for we have found a 27,000-member library that also confers protection against MP (results not shown). ELI should be readily applicable to viral pathogens, including HIV, considering that most have genomes that are 10- to 100-fold smaller than that of mycoplasma. □

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Increased T-cell apoptosis and terminal B-cell differentiation induced by inactivation of the Ets-1 proto-oncogene

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THE Ets-1 proto-oncogene is a member of a transcription factor family characterized by homology to the *v-ets* oncogene¹⁻⁴. In adult mice, Ets-1 is expressed predominantly in lymphoid cells where it has been implicated in regulating transcription of lymphocyte-specific genes⁵⁻⁷. Following T-cell activation, the specific DNA binding activity of Ets-1 is inactivated by transient phosphorylation, suggesting a function in the transition from the resting to activated state^{8,9}. Ets-1 has also been suggested to cooperate with the AP-1 transcription factor complex to mediate cellular growth factor responses⁴. Here we show, by using RAG-2-deficient blastocyst complementation¹⁰, that Ets-1 deficiency has dramatic, but different, effects on development and function of T- and B-lineage cells. Ets-1-deficient T cells were present in reduced numbers and were highly susceptible to cell death *in vitro*. In contrast, Ets-1-deficient B cells were present in normal numbers but a large proportion were IgM plasma cells. Our data demonstrate that Ets-1 is essential for maintenance of the normal pool of resting T- and B-lineage cells.

To investigate Ets-1 function in the immune system, we assayed the ability of Ets-1-deficient embryonic stem (ES) cells to differentiate into B and T lymphocytes following introduction into RAG-2^{-/-} blastocysts. In the resulting chimaeras, all peripheral B and T cells are derived from ES cells^{10,11}. We used gene-targeted mutation¹² to replace a region of the *Ets-1* gene^{13,15} encoding the DNA-binding domain (the last 110 amino acids of the protein) with a neomycin resistance gene (Fig. 1a). Heterozygous mutant (*Ets-1*^{+/-}) ES cells were selected for growth in high G418 concentrations to isolate homozygous mutant (*Ets-1*^{-/-}) ES cells¹⁶. *Ets-1*^{-/-}-RAG-2^{-/-} chimaeric mice derived from two independently targeted *Ets-1*^{-/-} ES cell clones contained substantial numbers of splenic B and T cells but severely reduced numbers of lymph-node lymphocytes. Ets-1-hybridizing transcripts were specifically lacking in RNA from

either total spleen cells (data not shown) or bacterial lipopolysaccharide (LPS)-induced B-cell blasts of *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeras, although their levels of immunoglobulin C_μ-hybridizing transcripts were similar to those of controls (Fig. 1b). Taken together, these data show that *Ets-1* expression was ablated by the introduced mutation, and that *Ets-1* expression is not absolutely required for generation of peripheral T and B cells.

At age 3–8 weeks, thymuses of *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeric mice had markedly reduced cellularity compared to those of *Ets-1*^{+/-}-*RAG-2*^{-/-} chimaeric or wild-type mice (Fig. 1c). Expression of the Ly9.1 marker¹⁷ was used to distinguish ES cell-derived from *RAG-2*^{-/-} thymocytes. *Ets-1*^{-/-} thymocyte populations had an increased proportion of immature cells, as indicated by increased representation of CD4⁺8⁺ cells (Fig. 2) and of CD25⁺ cells (data not shown). These data suggest either an impediment in T-cell development or increased turnover of more mature thymocytes. Splenic T-cell numbers in *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeras were reduced, on average, about threefold compared to those of *Ets-1*^{+/-}-*RAG-2*^{-/-} chimaeras or wild-type mice (Figs 1c and 2). Peripheral *Ets-1*^{-/-} T cells expressed normal levels of T-cell antigen receptor (TCR)-αβ complexes, but had reduced levels of CD5 and no detectable Thy-1 (data not shown).

Splenic B-lineage (B220⁺) cells were similar in number in *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeras and control mice (Fig. 1c). However, *Ets-1*^{-/-}-*RAG-2*^{-/-} B-cells contained a new population of

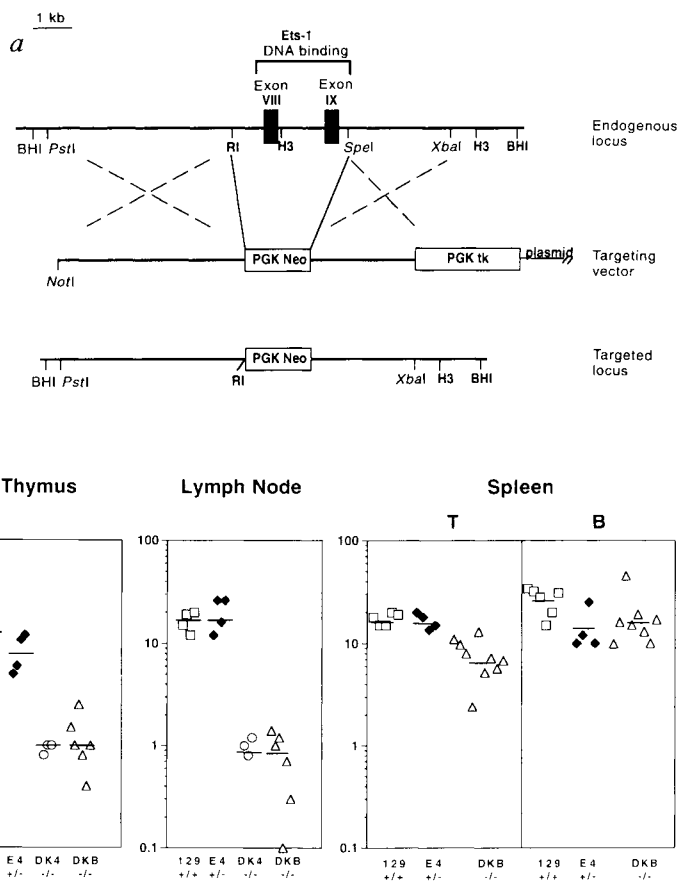
B220^{lo}/IgM⁺ cells which also comprised a new IgM⁺/IgD⁻ population (Fig. 2 and data not shown). In addition, a greater fraction of IgD⁺ B-lineage splenocytes expressed low levels of IgM in *Ets-1*^{-/-}-*RAG-2*^{-/-} versus control mice (Fig. 2), suggesting an increased proportion of more mature B cells¹⁸. Strikingly, plasma cells constituted between 19% and 34% of all splenic lymphoid cells in *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeras, as opposed to 2% or less in control mice (Fig. 3a, c). Most of these *Ets-1*^{-/-} plasma cells stained strongly for cytoplasmic IgM (Fig. 3b, d), correlating with dramatically elevated serum IgM levels in *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeras (Fig. 3e). Serum IgG1, IgG2a, IgG2b, IgG3 and IgE levels were normal in *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeras, and their splenic B cells could be induced to switch to these isotypes *in vitro*, indicating that class switching was not blocked (data not shown). Increased IgM levels and plasma cell numbers (along with reduced T-cell numbers) are also characteristics of the motheaten (*me/me*) mouse, which has a defective haematopoietic cell tyrosine phosphatase¹⁹. However, although most splenic B cells of *me/me* mice are CD5⁺ (ref. 20), those of *Ets-1*^{-/-} chimaeras, like those of normal mice, are primarily CD5⁻ (data not shown).

To assess whether the T-cell deficiency in *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeras was associated with impaired survival, purified splenic T cells were assayed for spontaneous cell death in culture using a flow cytometric assay. Whereas more than 75% of wild-type and *Ets-1*^{+/-} splenic T cells were alive at 48 hours, only 11% of *Ets-1*^{-/-} T cells survived under such conditions (Fig. 4a).

FIG. 1 Disruption of the murine *Ets-1* gene and reconstitution of the lymphoid system in *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeras. **a**, Restriction map of the 3' region of the murine *Ets-1* locus. Exons are indicated as black boxes and correspond to the human exons VIII and IX (ref. 22). Restriction sites: BHI, *Bam*HI; RI, *Eco*RI; H3, *Hind*III. The structure of the targeting vector and the mutated *Ets-1* gene are indicated. **b**, Northern blot analysis of total RNA from *Ets-1*^{-/-} or 129sv splenic lymphocytes stimulated with LPS (20 μg ml⁻¹) for 4 days. Left, the blot was hybridized with a 5' *Ets-1* complementary DNA probe. Right, the same blot was hybridized with an IgH C_μ probe. The mobilities of 18S and 28S ribosomal RNA bands are indicated. **c**, Cellularity of lymphoid organs from *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeras. Chimaeric mice were produced by injection of two independent *Ets-1*^{-/-} ES cell clones into *RAG-2*^{-/-} blastocysts. The numbers of cells derived from individual *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeras are indicated by open circles (clone DK4) or open triangles (clone DKB); *Ets-1*^{+/-}-*RAG-2*^{-/-} chimaeras are shown by filled diamonds (clone E4), and wild-type mice by open squares. Mean values are indicated by horizontal bars.

METHODS. **a**, A murine *Ets-1* genomic clone was isolated from a strain 129sv genomic library and the organization of exons characterized using standard procedures. Within a 12-kb *Pst*I–*Xba*I fragment, a 3-kb *Eco*RI–*Spe*I fragment containing the exon VIII and IX was replaced by a PGK-neo^r cassette. A PGK-tk cassette was inserted adjacent to the 3' flanking region. The construct was linearized by *Not*I digestion and transfected into J1 ES cells²³. G418 and gancyclovir resistant clones were screened by Southern blotting to identify homologous recombination events. *Ets-1*^{-/-} ES cells

were subjected to increased G418 concentration to select homozygous *Ets-1*^{-/-} clones²¹. Chimaeric mice were generated by injection of *Ets-1* targeted ES cells into *RAG-2*^{-/-} blastocysts¹⁰. To control for variability in ES cell contribution to the lymphoid lineage, peripheral blood of chimaeric mice was tested by flow cytometry using antibodies to CD4 and CD8, or B220 and IgM (see Fig. 2). Only mice that exhibited reconstitution of both T and B cells in the circulation were utilized for subse-



quent analysis. **b**, Northern blots were hybridized with a 1.5-kb *Eco*RI fragment corresponding to the 5' region of the cDNA. The blot was subsequently hybridized with a C_μ probe (*Xba*I–*Bam*HI genomic fragment)¹⁶. **c**, Cell suspensions were made by teasing lymphoid tissues over nylon mesh and counted. Splenic T- and B-cell fractions were determined using flow cytometry (Fig. 2).

Furthermore, DNA fragmentation was detected in the *Ets-1*^{-/-} T cells after 15 hours, indicating increased susceptibility to apoptosis (Fig. 4b); similar results were obtained for total thymocytes (data not shown). Addition of concanavalin A (conA) or ionomycin plus phorbol 12-myristate 12-acetate (PMA) did not rescue the survival defect or induce proliferation of *Ets-1*^{-/-} T-cell cultures as measured by ³H-thymidine incorporation; however, treatments with these agents, as well as anti-CD3 ϵ , did induce expression of early activation markers (CD69 and CD25), indicating that initial cell-surface signalling events were not impaired (data not shown).

No differences in survival between purified (B220⁺) wild-type and *Ets-1*^{-/-} splenic B cells were noted (Fig. 4a). In addition,

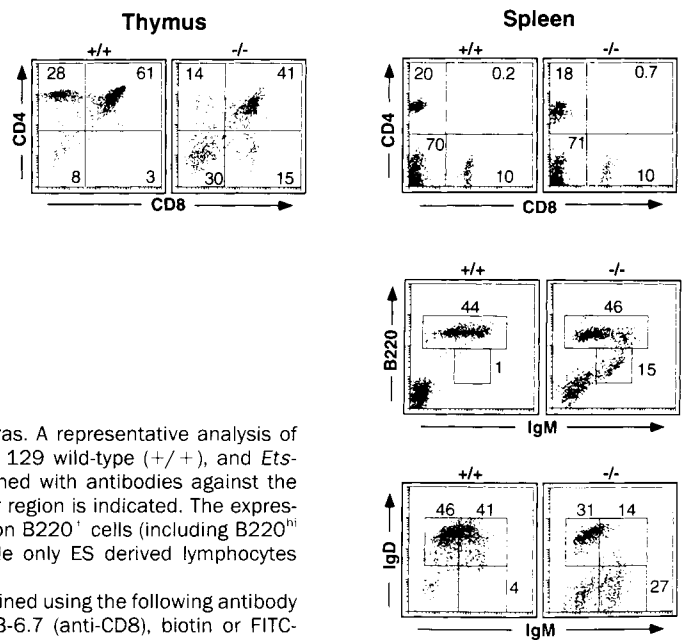


FIG. 2 Flow cytometric analysis of lymphoid cells from *Ets-1*^{-/-} chimaeras. A representative analysis of lymphocyte subsets present in the thymus and spleen of 3–4-week old 129 wild-type (+/+), and *Ets-1*^{-/-} RAG-2^{-/-} chimaeric (-/-) mice is shown. Lymphocytes were stained with antibodies against the surface antigens indicated. The percentage of events in each quadrant or region is indicated. The expression of IgM versus IgD on splenocytes was analysed by electronic gating on B220^{hi} cells (including B220^{hi} and B220^{lo} populations); all the other plots show data gated to include only ES derived lymphocytes expressing Ly9.1 (ref. 17).

METHODS. Single cell suspension of thymocytes and spleen cells were stained using the following antibody conjugates: phycoerythrin (PE)-H129-19 (anti-CD4), fluorescein (FITC) 53-6.7 (anti-CD8), biotin or FITC-30C7 (anti-Ly9.1), PE-RM2-5 (anti-CD2), FITC-3C7 (anti-CD25), PE-RA3.6B2 (anti-B220), FITC-R6-60.2 (anti-IgM), biotin-AMS 9.1 (anti-IgD^{hi}), and FITC-53-7.3 (anti-CD5). Biotinylated antibodies were detected with streptavidin-CyChrome. All antibodies were obtained from Pharmingen. Data were analysed using a FAC-Scan flow cytometer (Becton-Dickinson).

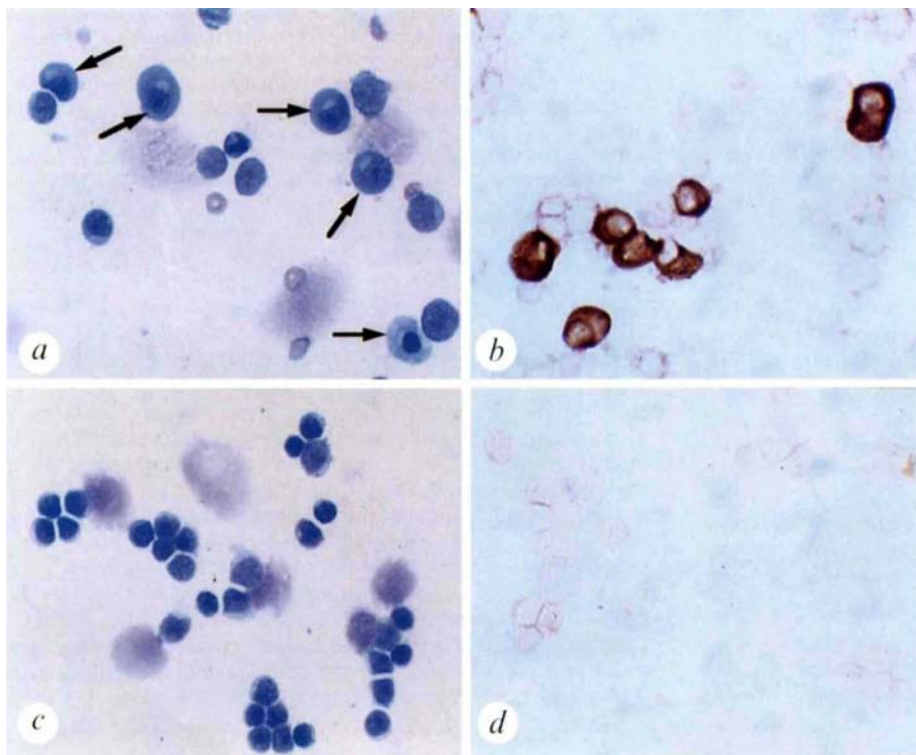
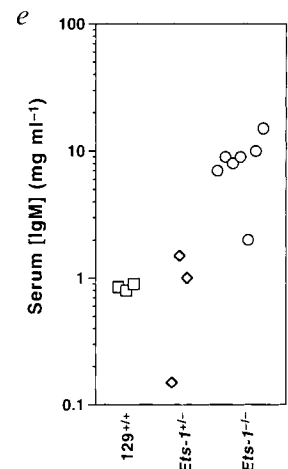


FIG. 3 Cytologic analyses of splenic lymphocytes. a, Cytologic preparation of spleen cells from a representative *Ets-1*^{-/-} RAG-2^{-/-} chimaera, demonstrating abundant plasma cells (arrows). b, Immunohistochemical stain for IgM from the same mice, showing plasma cells with cytoplasmic IgM. c, Spleen cytology from an *Ets-1*^{-/-} RAG-2^{-/-} chimaera, showing predominantly small lymphocytes. d, IgM stain on *Ets-1*^{-/-} splenocytes, which exhibited only weak membrane staining for IgM on a subset of cells. e, Concentration of serum in wild-type 129 mice (squares), *Ets-1*^{-/-} RAG-2^{-/-} chimaeras (diamonds) and *Ets-1*^{-/-} RAG-2^{-/-} chimaeras (circles). Each point corresponds to an individual chimaeric mouse; *Ets-1*^{-/-} RAG-2^{-/-} chimaeras were derived from clone DKB. **METHODS.** a–b, Spleen cells were deposited on poly-L-lysine-coated glass slides using a cytocentrifuge (330 r.p.m., 5 min). Slides were either

stained with Wright-Giemsa stain, or fixed in acetone and stained with biotin-conjugated anti-IgM (Pharmingen), followed by avidin-horseradish peroxidase (Extravidin, Sigma) and developed using Diaminobenzidine (Sigma) according to the manufacturer's recommendations. Plasma cells were identified by typical morphological appearance on Wright-Giemsa-stained preparations and quantified by differential counting of 200 consecutive lymphoid cells. e, Sera from 4–8-week-old 129 mice, *Ets-1*^{-/-} RAG-2^{-/-} and *Ets-1*^{-/-} RAG-2^{-/-} chimaeras were screened by enzyme-linked immunosorbent assay (ELISA) as described²³. Goat anti-mouse IgM antibodies were purchased from Southern Biotechnology Associates. The data shown are representative for three independent ELISA assays. Sera from RAG-2^{-/-} mice were included as negative controls in each assay (data not shown).



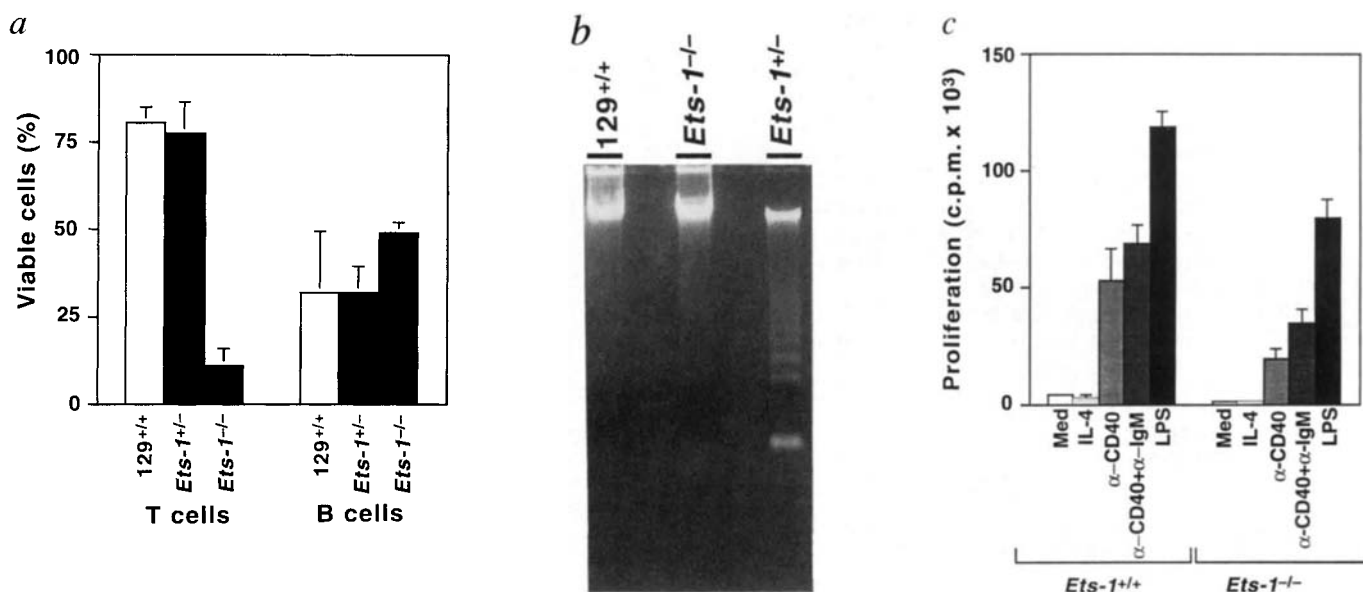


FIG. 4 Survival and proliferation of *Ets-1*-deficient lymphocytes. **a**, Survival of purified splenic T and B cells from wild-type mice, *Ets-1*^{+/-}-RAG-2^{-/-} and *Ets-1*^{-/-}-RAG-2^{-/-} chimaeras after 48 h in culture. **b**, DNA fragmentation following 15 h culture of splenic T cells from wild-type mice, *Ets-1*^{+/-}-RAG-2^{-/-} or *Ets-1*^{-/-}-RAG-2^{-/-} chimaeras. **c**, Proliferation of B220⁺ sorted B cells in medium alone, medium plus IL-4 or medium plus IL-4 supplemented with either Lipopolysaccharide (LPS), soluble anti-CD40 or soluble anti-CD40 plus soluble anti-IgM. Proliferation was measured after 48 h by ³H-thymidine incorporation. **METHODS.** **a**, Spleen cells from 129sv control mice and *Ets-1*^{-/-}-RAG-2^{-/-} chimaeras were stained with PE-anti-CD4 and PE-anti-CD8 for T cells and PE-B220 for B cells and purified by cell sorting (purity > 95%). Live and apoptotic cells were distinguished by forward light scatter (FSC) and side scatter (SSC)²⁴, either directly after sorting or after 48 h in culture in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U ml⁻¹ penicillin/streptomycin, 50 mg ml⁻¹ 2-mercaptoethanol.

Ets-1^{-/-} B cells proliferated significantly in response to LPS, anti-CD40, or anti-CD40 plus anti-IgM treatments, although responses appeared somewhat reduced (particularly for the latter two treatments; Fig. 4c). These reductions suggest a direct role for *Ets-1* in B-cell proliferation in the context of particular activation pathways; alternatively, the differential proliferative responses may simply reflect the novel subset composition of the splenic B cells in *Ets-1*^{-/-}-RAG-2^{-/-} mice.

Ets-1 has been linked to the regulation of IgH and TCR- α genes through binding to specific sites in their enhancers^{5,6}. However, expression of these genes still occurred in lymphocytes deficient in *Ets-1*, so it is possible that *Ets-1* binding is not required for such transcription or that it may be replaced in this context by another *Ets* family member⁶. In either case, our data demonstrate that *Ets-1* deficiency results in the inability to main-

tain a normal pool of resting immunocompetent T and B cells. However, *Ets-1* deficiency manifested differently in the T-cell versus B-cell compartment. Mature *Ets-1*-deficient T cells, as well as total *Ets-1*-deficient thymocytes, were highly susceptible to cell death. In contrast, there were relatively normal numbers of *Ets-1*-deficient B cells, but many showed abnormal differentiation towards IgM-secreting plasma cells. It remains possible that aspects of the phenotypic alterations observed in *Ets-1*^{-/-}-RAG-2^{-/-} chimaeras may not be intrinsic to the affected lymphocyte lineages. However, given that *Ets-1* expression in adult mice is predominantly confined to resting lymphocytes^{3,21}, we propose that the observed abnormalities reflect a function of *Ets-1* in actively maintaining lymphocytes in a resting, G0 cell-cycle stage. In this context, the consequences of escaping such negative regulation may activate default cell death pathways in T cells and favour terminal differentiation in B cells. □

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Defective activation and survival of T cells lacking the *Ets-1* transcription factor

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THE *Ets-1* proto-oncogene¹ is a member of the *Ets* family of eukaryotic transcription factors²⁻⁷. Members of this family play important roles in regulating gene expression in response to multiple developmental and mitogenic signals^{4,5,8,9}. *Ets-1* is preferentially expressed at high levels in B and T cells of adult mice^{10,11} and is regulated during both thymocyte development¹¹ and T-cell activation^{12,13}. To study the role of *Ets-1* in T-cell development and function we have used the *RAG-2*^{-/-} complementation system¹⁴ and murine embryonic stem (ES) cells containing homozygous deletions in the *Ets-1* gene (*Ets-1*^{-/-}). *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeric mice displayed markedly decreased numbers of mature thymocytes and peripheral T cells. *Ets-1*^{-/-} T cells expressed normal levels of CD3 and T-cell antigen receptor (TCR)- α/β . However, they displayed a severe proliferative defect in response to multiple activational signals and demonstrated increased rates of spontaneous apoptosis *in vitro*. These findings demonstrate that *Ets-1* is required for the normal survival and activation of murine T cells.

To assess directly the role of *Ets-1* in T-cell development and function, a null mutation was introduced into one (*Ets-1*^{+/-}) or both (*Ets-1*^{-/-}) copies of the murine *Ets-1* gene using homologous recombination (Fig. 1a). Two different ES cell clones containing homozygous mutations of the *Ets-1* gene were produced (Fig. 1b) and injected into *RAG-2*-deficient blastocysts¹⁵ to produce *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeric mice. Because mature B and T cells cannot develop in the absence of the *RAG-2* gene product, this system provides a stringent test of the role of *Ets-1* in lymphoid development and function¹⁴. Each mouse included in our analyses demonstrated significant although variable contributions of the *Ets-1*^{-/-} ES cells to multiple tissues as assayed by Southern blot analysis (Fig. 1c, d). Thymi from the *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeric mice were derived almost entirely from the *Ets-1*^{-/-} ES cells, thereby validating the usefulness of this system for determining the role of *Ets-1* in T-cell development and function. The lack of expression of the murine *Ets-1* protein of relative molecular mass (*M_r*) 60K in *Ets-1*^{-/-} ES cell-derived thymocytes was confirmed by immunoblot analysis (Fig. 1e).

Ets-1^{-/-}-*RAG-2*^{-/-} mice aged 8–12 weeks contained profoundly reduced numbers of thymocytes compared to age-matched wild-type (*Ets-1*^{+/+}) mice or control chimaeric mice produced by injection of *Ets-1*^{+/+} ES cells into *RAG-2*^{-/-} blastocysts (*Ets-1*^{+/+}) (Table 1). As reported previously¹⁵, thymi from the *RAG-2*^{-/-} animals contained small numbers of thymocytes which were shown by flow cytometric analyses to be composed almost exclusively of immature CD4⁺/CD8⁻ double-negative (DN) cells (Fig. 2a). In contrast, thymi from the *Ets-1*^{-/-}-*RAG-2*^{-/-} mice contained significant numbers of more mature CD4⁺/CD8⁺ double-positive (DP) as well as CD4⁺ and CD8⁺ single-positive (SP) cells (Fig. 2a). Southern blot analyses demonstrated that, as expected¹⁵, the *RAG-2*^{-/-} thymocytes had not undergone TCR- β gene rearrangements. In contrast, thymocytes from the *Ets-1*^{-/-}-*RAG-2*^{-/-} mice displayed normal levels of TCR- β gene rearrangement (data not shown).

Despite the finding of DP and SP thymocytes in the *Ets-1*^{-/-}-*RAG-2*^{-/-} mice, there was a reproducible and significant

TABLE 1 Lymphocyte populations in *Ets-1*^{-/-}-*RAG-2*^{-/-} chimaeric mice

	<i>RAG-2</i> ^{-/-} (n = 3)	Cell number ($\times 10^6$) <i>Ets-1</i> ^{+/+} (n = 4)	<i>Ets-1</i> ^{+/-} (n = 2)	<i>Ets-1</i> ^{-/-} (n = 4)
Thymus	8.7 $\pm$ 0.49	79 $\pm$ 13	32	8.8 $\pm$ 1.7
Spleen				
Total	9.3 $\pm$ 2.7	80 $\pm$ 6.6	40	12 $\pm$ 2.6
CD3 ⁺	0.14 $\pm$ 0.06	13.12 $\pm$ 3.12	3.38	0.88 $\pm$ 0.01
IgM ⁺	0.19 $\pm$ 0.14	12.66 $\pm$ 1.8	3.18	2.23 $\pm$ 0.08

Viable lymphocytes from thymi, and spleens of *RAG-2*^{-/-}, wild type (*Ets-1*^{+/+}), *Ets-1*^{+/-}-*RAG-2*^{-/-} (*Ets-1*^{+/-}), or *Ets-1*^{-/-}-*RAG-2*^{-/-} (*Ets-1*^{-/-}) chimaeric mice were quantitated by trypan blue exclusion and manual counting on a haemocytometer. Splenic lymphocytes were stained with the following antibody conjugates: FITC-labelled 145-2C11 (anti-CD3 ϵ), or FITC-labelled R40-97 (anti-IgM) and analysed by flow cytometry using a Becton-Dickinson FACScan and LYSYS II software. The data are shown as mean $\pm$ s.e.m.

increase in the relative numbers of immature DN cells and a concomitant decrease in the proportion of more mature DP thymocytes in these animals compared to either wild-type mice or control *Ets-1*^{+/+}-*RAG-2*^{-/-} chimaeric mice (Fig. 2a). This may reflect a specific block in the development of DN cells or increased turnover or death of the DP cells. The relative proportions of mature CD4⁺ and CD8⁺ SP thymocytes were normal in the *Ets-1*^{-/-}-*RAG-2*^{-/-} mice, despite severe reductions in the absolute numbers of these cells (Fig. 2a). Moreover, these SP thymocytes expressed normal levels of cell-surface CD3 and TCR- α/β (data not shown). In contrast, the *Ets-1*^{-/-} thymocytes displayed a small but reproducible reduction in cell-surface CD8 expression (Fig. 2a). Thus *Ets-1* is not required for thymocyte maturation, but is required to generate and/or maintain normal numbers of mature DP and SP thymocytes.

Total numbers of splenic and lymph-node T cells were also significantly reduced in the *Ets-1*^{-/-}-*RAG-2*^{-/-} mice (Table 1). However, both spleens and lymph nodes from these animals contained normal proportions of mature SP CD4⁺ and CD8⁺ T cells (Fig. 2b, c). The SP splenic T cells from the *Ets-1*^{-/-}-*RAG-2*^{-/-} animals expressed normal levels of cell-surface CD3 and TCR- α/β and slightly reduced levels of CD8 (Fig. 2b). The total numbers of IgM⁺ cells in the spleens from the *Ets-1*^{-/-}-*RAG-2*^{-/-} mice were similar to those of the *Ets-1*^{+/+}-*RAG-2*^{-/-} control mice (Table 1). However, spleens from the *Ets-1*^{-/-}-*RAG-2*^{-/-} mice contained significantly more IgM⁺/B220^{high} cells, and significantly fewer IgM⁺/B220⁺ B cells than either the *Ets-1*^{+/+}-*RAG-2*^{-/-} or the wild-type control spleens (Fig. 2b). These findings might reflect increased numbers of plasma cells in the spleens of the *Ets-1*^{-/-}-*RAG-2*^{-/-} mice (J.-C. Bories and F. Alt, personal communication) or a defect in the maturation or survival of mature *Ets-1*^{-/-} B cells.

That profoundly decreased numbers of thymocytes and peripheral T cells were found in the *Ets-1*^{-/-}-*RAG-2*^{-/-} mice suggested either a significant defect in proliferation and/or increased turnover of the *Ets-1*^{-/-} T cells *in vivo*. Purified splenic T cells from the *Ets-1*^{-/-}-*RAG-2*^{-/-} mice displayed a severe defect in proliferation in response to activation by crosslinking of the antigen receptor with an α -CD3 monoclonal antibody or by treatment with concanavalin A (conA) (Fig. 3a and data not shown). Similar proliferative defects were observed in *Ets-1*^{-/-} thymocytes activated by antigen receptor crosslinking or by treatment with conA (data not shown). In contrast, splenic B cells from the *Ets-1*^{-/-}-*RAG-2*^{-/-} animals proliferated normally in response to stimulation with lipopolysaccharide (LPS) (Fig. 3b).

Both thymocytes and purified splenic T cells from the *Ets-1*^{-/-}-*RAG-2*^{-/-} mice also displayed significantly higher rates of cell death than control *Ets-1*^{+/+} T cells during *in vitro* culture in the absence of an activating signal (Fig. 3c). After

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30 h in culture, less than 25% of the *Ets-1*^{-/-} thymocytes remained viable, compared with more than 55% of the *Ets-1*^{+/+} cells ($P < 0.001$). Similarly, 24% of the *Ets-1*^{-/-} splenic T cells were viable after 36 h in culture, compared with 47% of the *Ets-1*^{+/+} T cells ($P < 0.002$). This decreased cell survival was associated with a significantly increased rate of apoptosis of the *Ets-1*^{-/-} T cells (Fig. 3d). After 30 h in culture, 58% of the *Ets-1*^{-/-} thymocytes had undergone apoptosis, compared with 33% of control *Ets-1*^{+/+} thymocytes (Fig. 2d). These results demonstrate a severe defect in proliferation and a significantly enhanced susceptibility to apoptotic cell death in *Ets-1*^{-/-} thymocytes and peripheral T cells.

Previous studies^{7,16,19} have identified functionally important *Ets-1* binding sites in the transcriptional regulatory regions of several T-cell-specific genes including the TCR- α and TCR- β enhancers, and the CD4 promoter. Our results demonstrate that

Ets-1 is not required for the expression of these genes during T-cell development. This may reflect either the relative unimportance of *Ets-1* in regulating these genes or, more likely, the fact that other *Ets* family members expressed in T cells can substitute for *Ets-1* in regulating gene expression during T-cell differentiation. In contrast, our results demonstrate that *Ets-1* is important for regulating proliferative and apoptotic pathways in T cells, and may also be important in regulating normal B-cell maturation. *Ets-1* is first expressed on days 17 and 18 of murine embryonic development, when mature SP thymocytes begin to accumulate in large numbers^{10,11,20}. Moreover, functional *Ets-1* protein is expressed at high levels in mature quiescent T cells^{10,11}, but is efficiently inactivated and degraded after T-cell activation^{12,13,21}. When taken together with our results, these findings are consistent with a model in which *Ets-1* is required to maintain mature T cells in phase G0 of the cell cycle. In such

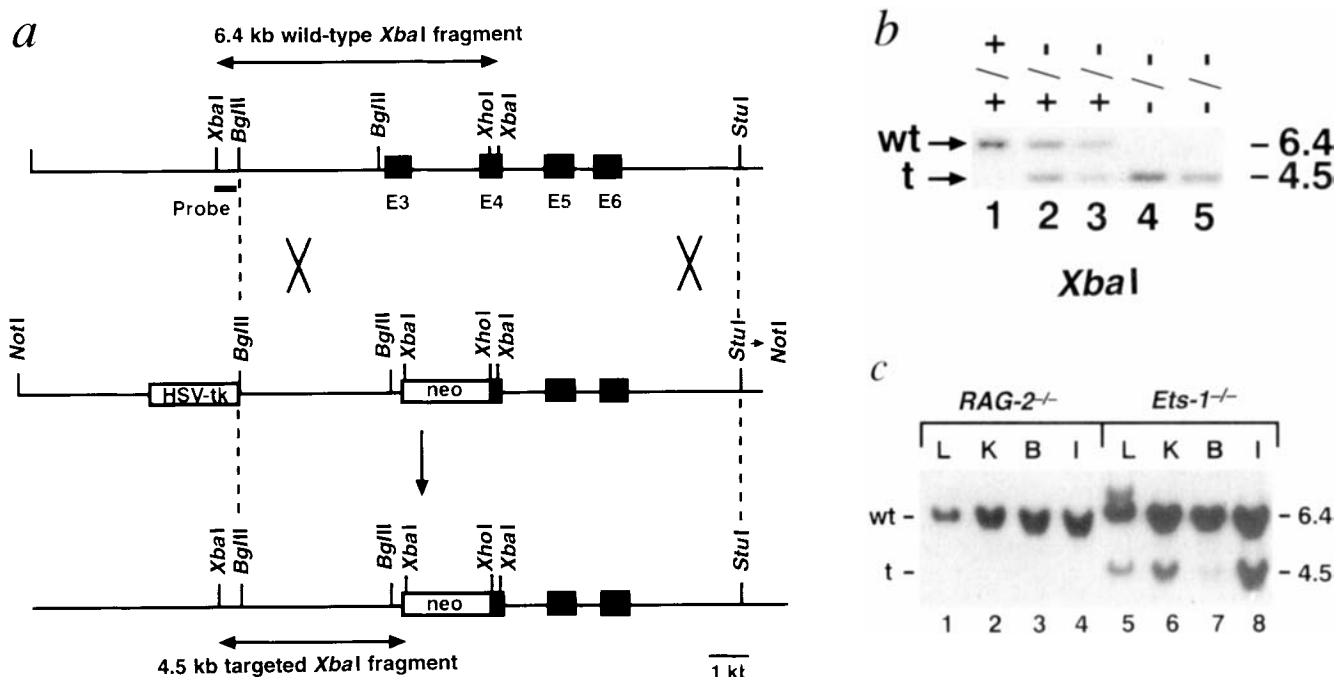


FIG. 1 Targeted disruption of the *Ets-1* gene. a, Schematic representation of the *Ets-1* targeting construct. Top, a partial restriction endonuclease map of the murine *Ets-1* gene; middle, the structure of the *Ets-1* targeting construct containing the HSV-tk and neomycin resistance (*neo*) genes both under the control of the PGK promoter; bottom, the structure of the targeted *Ets-1* allele. b, Southern blot analysis of wild type (+/+), heterozygous (+/-) and homozygous (-/-) mutant *Ets-1* ES cell clones. The 6.4-kb wild-type (wt) and 4.5-kb targeted (t) alleles are shown by arrows. c, d, Southern blot analyses of DNA from, in c, liver (L), kidney (K), brain (B), intestine (I), and d, thymi of RAG-2^{-/-} and *Ets-1*^{-/-}-RAG-2^{-/-} (*Ets-1*^{-/-}) chimaeric mice. e, Immunoblot analysis of *Ets-1* expression in thymocytes from wild-type (*Ets-1*^{+/+}) and *Ets-1*^{-/-}-RAG-2^{-/-} (*Ets-1*^{-/-}) mice. Size markers (M_r (K)) are shown to the right of the autoradiogram. The presence of equivalent levels of low- M_r nonspecific bands on the immunoblot confirmed that equal amounts of protein were loaded in both lanes of the gel.

METHODS. A murine genomic *Ets-1* clone was isolated from a 129sv genomic library (Stratagene) and characterized by restriction endonuclease and DNA sequence analysis. The targeting construct was produced by insertion of a 4.4-kb *Bgl*II fragment containing intron II of the *Ets-1* gene into the *Bam*HI site of pPNT (ref. 22) followed by ligation of a 6.0-kb *Xho*I/*Stu*I fragment of the *Ets-1* gene containing part of exon 4 and exons 5 and 6 into the *Not*I/*Xho*I sites of pPNT. The resulting vector was electroporated into CCE ES cells and transfectants were selected by growth in G418 and ganciclovir²³. ES cell clones and tissues from chimaeric mice were characterized by Southern blot analysis using *Xba*I-digested DNA and the 0.9-kb radiolabelled *Xba*I/*Bgl*II probe shown in a (Probe). Homozygous *Ets-1* mutant ES cell clones were generated

by selection of the *Ets-1*^{-/-} ES cells in 1,200–1,500 μ g ml⁻¹ of G418 (ref. 24). *Ets-1*^{+/+} ES cell clones used to produce control animals were also subjected to the high G418 selection but failed to be converted to the *Ets-1*^{-/-} genotype. Immunoblots containing whole-cell extracts from 2×10^6 thymocytes from wild-type (*Ets-1*^{+/+}) or *Ets-1*^{-/-}-RAG-2^{-/-} animals were probed with the C-20 polyclonal rabbit anti-*Ets-1* antiserum (Santa Cruz) (2 μ g ml⁻¹).

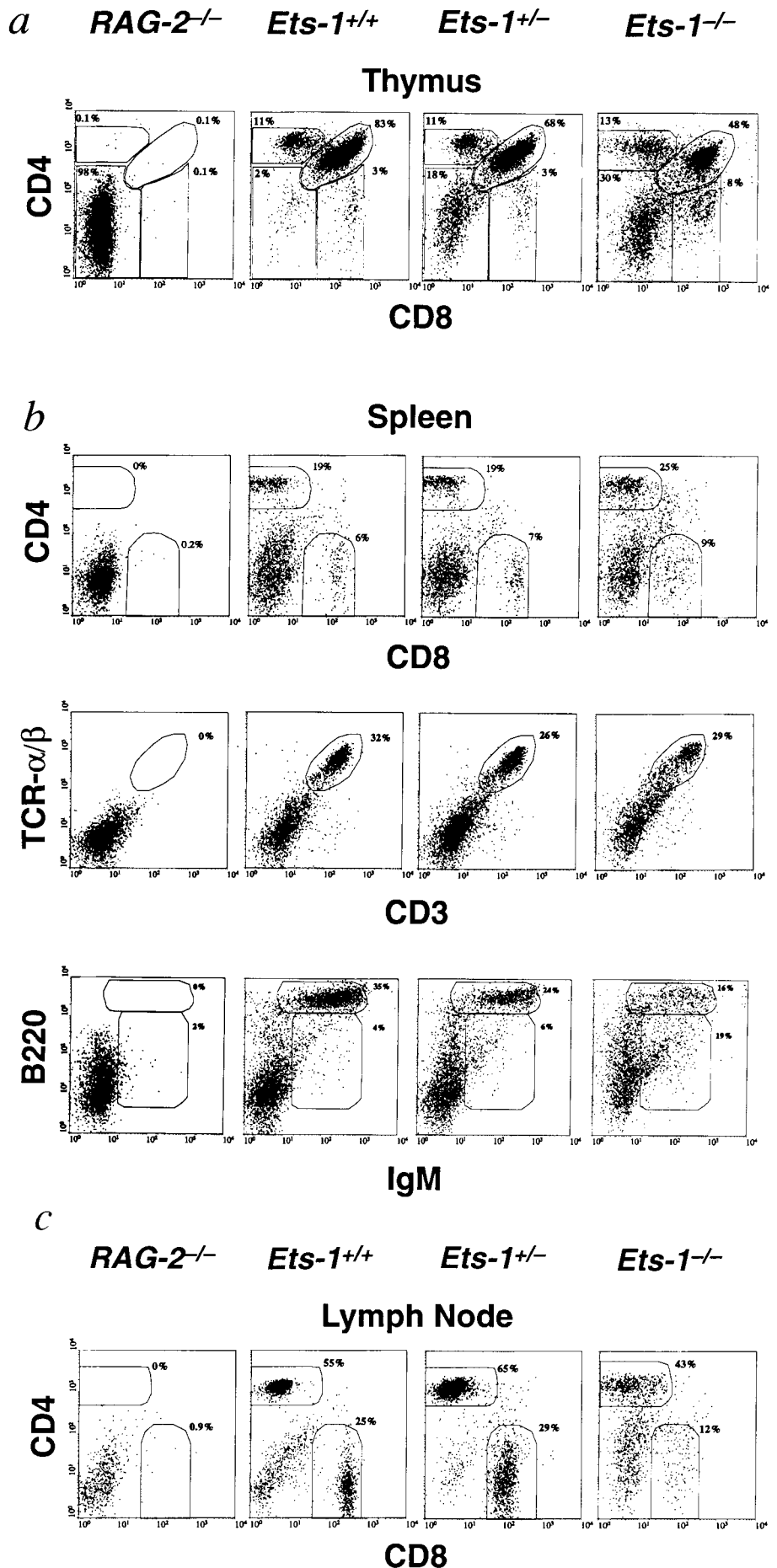


FIG. 2 Flow cytometric analyses of lymphocytes from *Ets-1*^{-/-}*RAG-2*^{-/-} chimaeric mice. Fluorescence staining profiles of lymphocytes from thymus (a), spleen (b) and lymph node (c) of *RAG-2*^{-/-}, wild-type (*Ets-1*^{+/+}) and *Ets-1*^{+/-}*RAG-2*^{-/-} (*Ets-1*^{+/-}) or *Ets-1*^{-/-}*RAG-2*^{-/-} (*Ets-1*^{-/-}) chimaeric mice. Each plot is representative of at least five mice (3–12 weeks old) of each type, except the *Ets-1*^{+/-} plot which is representative of two mice. Identical results were obtained from *Ets-1*^{-/-}*RAG-2*^{-/-} mice derived from two different *Ets-1*^{-/-} ES cell clones.

METHODS. Single-cell suspensions of lymphocytes were prepared and stained with the following antibody conjugates: phycoerythrin (PE)-labelled RM-4-5 (anti-CD4), fluorescein (FITC)-labelled 53-6.7 (anti-CD8a), PE-labelled H57-597 (anti-TCR-α/β), FITC-labelled 145-2C11 (anti-CD3ε), PE-labelled RA3-6B2 (anti-B220), FITC-labelled R40-97 (anti-IgM) (Pharmingen). Each plot represents analysis of more than 10⁴ events collected as listmode files using a FACScan flow cytometer (Becton-Dickinson) and analysed using LYSYS II software.

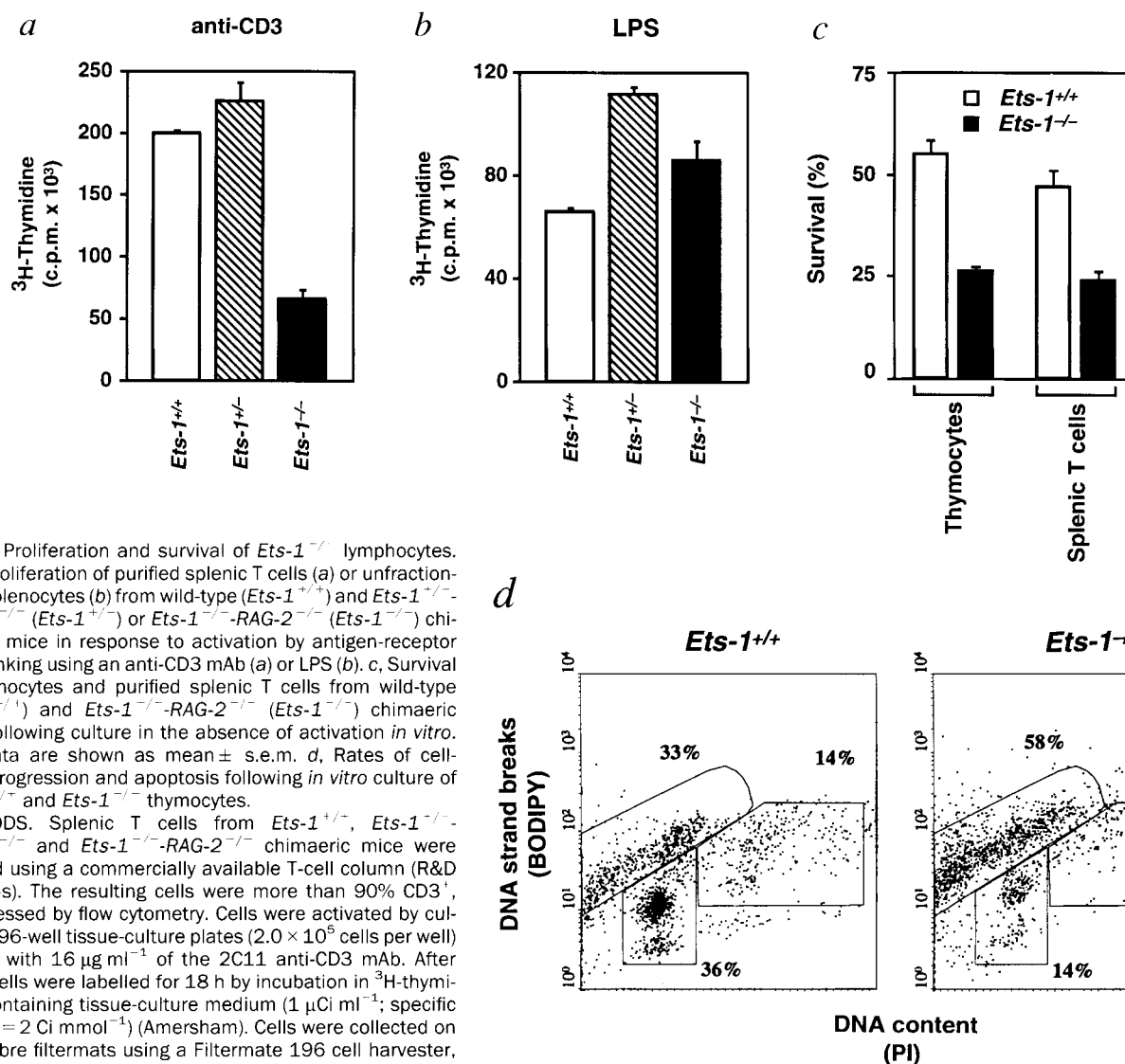


FIG. 3 Proliferation and survival of *Ets-1*^{-/-} lymphocytes. **a, b**, Proliferation of purified splenic T cells (**a**) or unfractionated splenocytes (**b**) from wild-type (*Ets-1*^{+/+}) and *Ets-1*^{-/-}-RAG-2^{-/-} (*Ets-1*^{+/+}) or *Ets-1*^{-/-}-RAG-2^{-/-} (*Ets-1*^{-/-}) chimaeric mice in response to activation by antigen-receptor crosslinking using an anti-CD3 mAb (**a**) or LPS (**b**). **c**, Survival of thymocytes and purified splenic T cells from wild-type (*Ets-1*^{+/+}) and *Ets-1*^{-/-}-RAG-2^{-/-} (*Ets-1*^{-/-}) chimaeric mice following culture in the absence of activation *in vitro*. The data are shown as mean ± s.e.m. **d**, Rates of cell-cycle progression and apoptosis following *in vitro* culture of *Ets-1*^{+/+} and *Ets-1*^{-/-} thymocytes.

METHODS. Splenic T cells from *Ets-1*^{+/+}, *Ets-1*^{-/-}-RAG-2^{-/-} and *Ets-1*^{-/-}-RAG-2^{-/-} chimaeric mice were purified using a commercially available T-cell column (R&D Systems). The resulting cells were more than 90% CD3⁺, as assessed by flow cytometry. Cells were activated by culture in 96-well tissue-culture plates (2.0 × 10⁵ cells per well) coated with 16 µg ml⁻¹ of the 2C11 anti-CD3 mAb. After 48 h, cells were labelled for 18 h by incubation in ³H-thymidine-containing tissue-culture medium (1 µCi ml⁻¹; specific activity = 2 Ci mmol⁻¹) (Amersham). Cells were collected on glass-fibre filtermats using a Filtermate 196 cell harvester, and ³H-thymidine incorporation was measured in a Top-count microplate scintillation counter (Packard). For assessment of B-cell activation, splenocytes were cultured for 48 h in medium containing LPS (50 µg ml⁻¹), and ³H-thymidine incorporation was determined as described above. All proliferation assays were performed in triplicate. For assays of cell survival, cell-cycle progression and apoptosis, thymocytes or purified splenic T cells from *Ets-1*^{+/+} and *Ets-1*^{-/-}-RAG-2^{-/-}

chimaeric mice were cultured in DMEM + 10% fetal bovine serum *in vitro* for 30 h (thymocytes) or 36 h (splenic T cells) (4 × 10⁶ cells in 2 ml of tissue culture medium in a 24-well plate). Cell viabilities were determined manually in triplicate by trypan blue exclusion. Cells were assayed for apoptosis and cell-cycle progression using propidium iodide and BODIPY-dUTP in conjunction with TdT in a single-step method²⁵.

a model the absence of Ets-1 might lead to abnormal cell-cycle progression, programmed cell death and concomitant T-cell depletion *in vivo*. Alternatively, it is possible that Ets-1 regulates the expression of genes such as *mur77*, *bel-2*, *bel-x* or *ICE* that control spontaneous or stress-induced apoptosis in T cells. □

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Membrane-restricted regulation of Ca^{2+} release and influx in polarized epithelia

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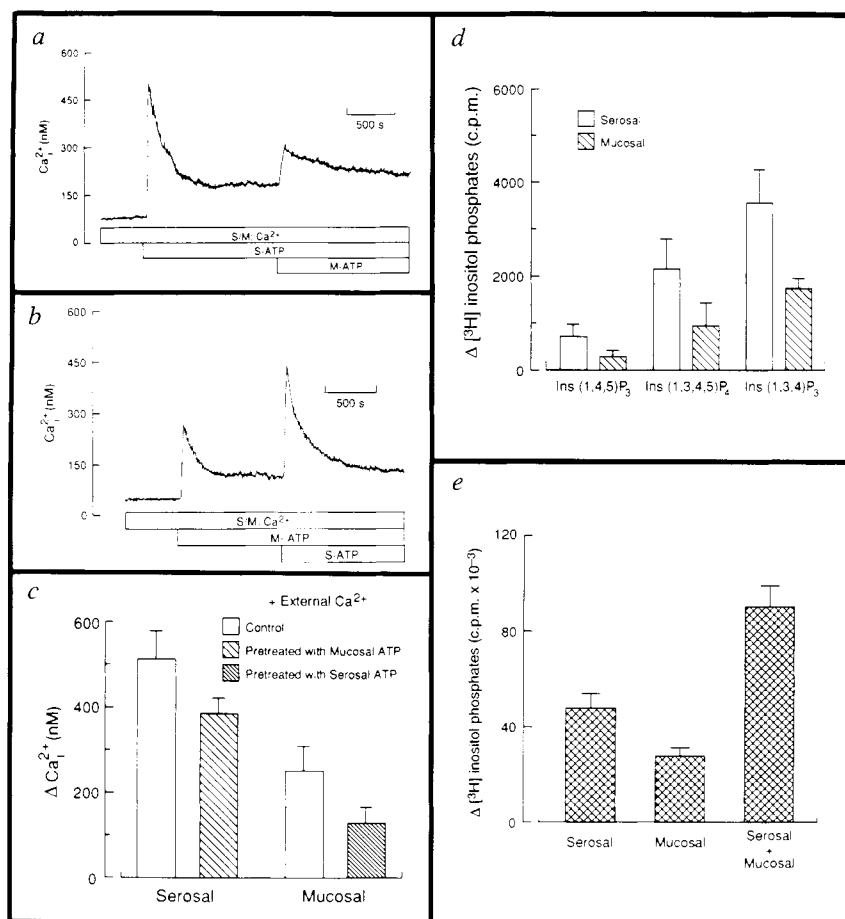
EPITHELIAL cells exist in a complex setting in which responses to mucosal or serosal environments are mediated by receptors expressed on specialized cellular domains, such as apical versus basolateral cell membranes. We investigated whether airway epithelia can react selectively through G-protein-coupled receptors to stimuli in the mucosal or serosal environments by measuring inositol phosphate and intracellular Ca^{2+} responses in polarized human nasal epithelial monolayers. We report here that unilateral ATP (10^{-4} M) administration stimulated P_2 purinoceptors and tapped pools of intracellular Ca^{2+} associated with the plasma membrane ipsilateral but not contralateral to stimulated receptors. Similarly,

activation of plasma membrane Ca^{2+} influx by ATP was confined to the membrane ipsilateral to receptor stimulation. These findings demonstrate that polarized epithelia restrict P_2 receptor-mediated responses to a single domain of the cell, reflecting membrane-specific generation and catabolism of inositol phosphates and confinement of calcium influx regulation to the membrane ipsilateral to the stimulated receptors.

The human nasal epithelial (HNE) cell preparation is a polarized monolayer by morphological and electrical criteria^{1,2}. During bilateral perfusion with Ca^{2+} -containing NaCl Ringer solutions, HNE monolayers were exposed to ATP (1) on the serosal (first addition) followed by the mucosal surface (second addition; Fig. 1a), or conversely (2) on the mucosal (first addition) followed by the submucosal surface (second addition; Fig. 1b). Analysis of the pattern of responses revealed (1) peak (transient) changes in intracellular calcium (Ca_i^{2+}) that are about twofold greater in response to serosal ATP as compared to mucosal ATP addition (Fig. 1c); and (2) sustained (plateau) phases that were also larger for serosal as compared to mucosal additions of ATP (Fig. 1 legend). High-performance liquid chromatography (HPLC) analysis of the [^3H]inositol phosphates generated by monolayers 30 s after incubation with ATP (Fig. 1d) showed ~twofold greater formation of inositol (1,4,5) trisphosphate ($\text{Ins}(1,4,5)\text{P}_3$) and its metabolites $\text{Ins}(1,3,4)\text{P}_3$ and inositol (1,3,4,5) tetrakisphosphate ($\text{Ins}(1,3,4,5)\text{P}_4$) after serosal

FIG. 1 Effects of serosal (S) and/or mucosal (M) ATP ($100 \mu\text{M}$) on intracellular Ca^{2+} (Ca_i^{2+}) in polarized HNE cells perfused bilaterally with NaCl Ringer solutions containing Ca^{2+} (1.3 mM). a, Response of Ca_i^{2+} (field size, 30 to 40 cells) to ATP applied sequentially to the serosal (S-ATP) and mucosal (M-ATP) bathing media. b, The response of Ca_i^{2+} to the sequential addition of mucosal (M-ATP) and serosal (S-ATP) ATP. c, Summarized data showing initial change in Ca_i^{2+} (ΔCa_i^{2+} , peak-basal value) in naive monolayers (control) first exposed to serosal ($n=6/3$ individuals) or mucosal ($n=6/3$ individuals) ATP, and then subsequently exposed to ATP on the contralateral membrane. In Ca^{2+} -containing Ringer, basal Ca_i^{2+} was $73 \pm 4 \text{ nM}$ ($n=58/8$ individuals). In comparison to this value, the sustained segments (plateaux) determined 15 min after the initial transient portion in naive monolayers exposed to serosal and mucosal ATP were $216 \pm 17 \text{ nM}$ ($n=6/3$ individuals) and $135 \pm 8 \text{ nM}$ ($n=6/3$ individuals), respectively. These plateaux are significantly different from each other and from basal values ($P<0.05$, Student's *t*-test). d, Formation of inositol (1,4,5) trisphosphate ($\text{Ins}(1,4,5)\text{P}_3$), $\text{Ins}(1,3,4)\text{P}_3$, and inositol (1,3,4,5) tetrakisphosphate ($\text{Ins}(1,3,4,5)\text{P}_4$) after a 30 s exposure to serosal or mucosal ATP ($n=3$ individuals). e, Total inositol phosphate accumulation following a 5 min exposure of serosal, mucosal or bilateral ATP ($n=3$ individuals). For d and e, data are c.p.m. expressed as total cellular radioactivity after subtracting background in the presence of Li^+ . All data are expressed as mean $\pm$ s.e.m.

METHODS. Cells were collected from human nasal tissue and plated on porous Transwell Col filters (pore diameter, $0.45 \mu\text{m}$; Costar) affixed to O-rings, maintained in hormone-supplemented Ham's F-12 medium¹¹, and studied at 10–12 days after seeding, a time coincident with the development of the maximal transepithelial potential difference $-10.6 \pm 4 \text{ mV}$, $n=78/8$ individuals, mean $\pm$ s.e.m.). For Ca_i^{2+} studies, HNE cell monolayers were loaded with Fura-2 and mounted in a miniature chamber¹² over an objective of a microscope coupled to a microfluorimeter. The fluorescence intensity ratio (excitation 340/380; emission $\geq 450 \text{ nm}$) was collected from a field of 30–40 HNE cells (see legends to Figs 1–3), or from a single cell (see legend to Fig. 4), and converted to Ca_i^{2+} as previously described¹³. ATP ($100 \mu\text{M}$) was used



at its maximally effective concentration as previously determined². For the inositol phosphate studies, HNE cells were plated on porous Transwell Col filters (diameter, 6.5 mm), and maintained under identical culture conditions as described above. Inositol lipid pools were labelled with [^3H]-myo-inositol, and stimulated products ($\pm\text{Li}^+$) resolved by HPLC as previously described¹⁴.

ATP as compared with mucosal ATP, and the sum of the products of serosal and mucosal ATP additions was additive (Fig. 1e). These data indicate that both apical and basolateral membranes express phospholipase C-coupled P_2 purinoceptors^{1,2}, and that P_2 receptor number/efficiency may be greater on the basolateral surface as compared to the apical border.

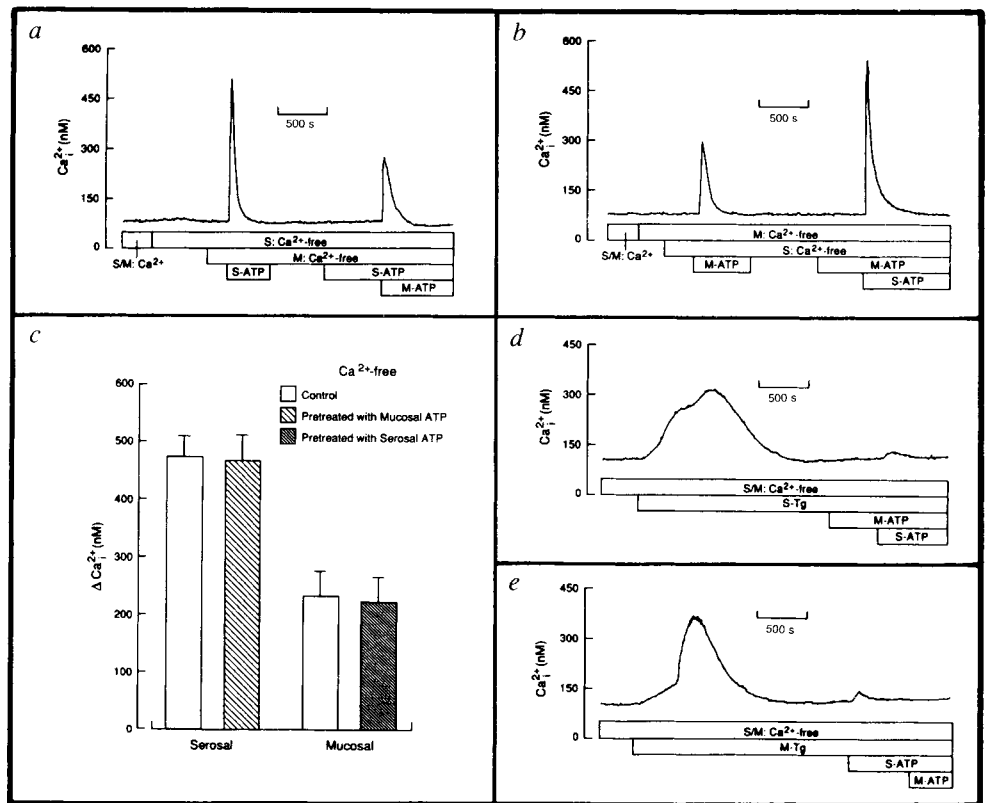
The magnitude of peak Ca_i^{2+} responses to the second ATP addition, that is, the response following ATP addition to the contralateral membrane, was reduced compared to the first addition responses (Fig. 1c), suggesting 'cross-talk' between membranes. Mechanisms that could reduce a contralateral-membrane response include receptor desensitization, tapping of contralateral internal Ca^{2+} stores by diffusable $Ins(1,4,5)P_3$ (ref. 3), and/or suppression of Ca^{2+} release from $Ins(1,4,5)P_3$ -sensitive pools by elevated Ca_i^{2+} (refs 4, 5). To distinguish between these possibilities, we did experiments in which HNE cell monolayers were bilaterally perfused with nominally Ca^{2+} -free Ringer solution. Under these conditions, the magnitude of the second ATP-induced Ca_i^{2+} response is not reduced by a previous exposure of the contralateral membrane to ATP (Fig. 2a-c). Note that, in contrast to studies with external Ca^{2+} (Fig. 1a,b), the levels of Ca_i^{2+} had returned to basal values at the time of the second addition of ATP (Fig. 2a,b). In contrast, the microsomal Ca^{2+} -ATPase inhibitor thapsigargin (Tg) accessed all internal Ca^{2+} pools when applied to either surface of HNE cell monolayers as revealed by the absent or reduced Ca_i^{2+} responses to additions of ATP ipsilateral or contralateral to Tg administration (Fig. 2d,e). These data demonstrate that unilateral ATP stimulation of P_2 receptors, but not Tg, results in release of Ca^{2+} from stores associated with the stimulated membrane but not the contralateral membrane (Fig. 2), suggesting that $Ins(1,4,5)P_3$ is generated locally but does not diffuse, probably reflecting local

degradation, from the stimulated to the contralateral membrane. The finding that the second ATP response is dampened by contralateral P_2 receptor activation in the presence of extracellular Ca^{2+} (Fig. 1c) implies that Ca^{2+} moves from the stimulated to the contralateral membrane.

We next tested whether activation of the Ca^{2+} influx pathway(s) is confined to the membrane containing the stimulated cell-surface receptor, or whether 'cross-talk' between membranes occurs. For these studies, the basolateral or apical membrane-associated Ca^{2+} stores were depleted by prestimulating with ATP under Ca^{2+} -free conditions, followed by selective exposure of the contralateral and stimulated membranes to extracellular Ca^{2+} (1.3 mM). Application of ATP to either the basolateral (Fig. 3a) or apical (Fig. 3b) membrane of HNE monolayers resulted in selective Ca^{2+} influx associated with the stimulated membrane (Fig. 3 legend). These Ca^{2+} influx responses persisted for 60 min in the presence of ATP, suggesting that the influx pathway(s) was not deactivated during this time period. Experiments in which ATP was administered as a 'pulse' and then removed 45 min before assessment of the Ca^{2+} influx path showed a partial deactivation of the influx pathway (Fig. 3c,d), suggesting a contribution of receptor occupancy to the activation state of the influx path (Fig. 3 legend). Measurement of inositol phosphates revealed return of inositol phosphate metabolism to baseline within 25 min of ATP removal (Fig. 3e).

Much evidence⁶ suggests that depletion of $Ins(1,4,5)P_3$ -sensitive Ca^{2+} stores is a key regulatory step for Ca^{2+} influx across the plasma membrane. Recent studies⁷ suggest that depletion of internal Ca^{2+} stores causes release of a soluble Ca^{2+} influx factor (CIF) from the same internal organelles from which Ca^{2+} was released. Our data suggest that if there is a soluble 'factor' liberated in response to depletion of internal Ca^{2+} stores, its effect/

FIG. 2 Effects of serosal (S) and/or mucosal (M) ATP (100 μ M) or thapsigargin (Tg, 500 nM) on intracellular free Ca^{2+} (Ca_i^{2+} , field size, 30–40 cells) in HNE cells bilaterally perfused with nominally Ca^{2+} -free (<10 μ M) NaCl Ringer solutions. a, Ca_i^{2+} measurement in a monolayer perfused bilaterally with Ringer solutions containing Ca^{2+} (1.3 mM; S/M Ca^{2+}) and then Ca^{2+} -free in the serosal (S: Ca^{2+} -free) and mucosal (M: Ca^{2+} -free) bathing media. Extracellular ATP was applied sequentially to the serosal (S-ATP) and mucosal (M-ATP) perfusate as indicated in the trace ($n=6/3$ individuals). b, Ca_i^{2+} measurements in HNE monolayers using a protocol identical to that described in trace a except that Ca^{2+} was removed first from the mucosal perfusate before changing to bilateral Ca^{2+} -free Ringer, and ATP was added to the mucosal bath before serosal ATP ($n=6/3$ individuals). c, Summarized data showing transient change in Ca_i^{2+} (ΔCa_i^{2+} , peak-basal value) in naive (control; serosal $n=20/5$ individuals, mucosal $n=20/5$ individuals) monolayers and in monolayers pretreated with serosal ($n=6/3$ individuals) or mucosal ($n=6/3$ individuals) ATP before activation of the P_2 receptors localized on the contralateral membrane. In monolayers bilaterally perfused in nominally Ca^{2+} -free NaCl Ringer solutions, basal Ca_i^{2+} was 68 ± 5 nM ($n=46/8$ individuals), a value not different ($P>0.05$, Student's *t* test) from the basal Ca_i^{2+} level in monolayers perfused with Ca^{2+} -containing Ringer solutions. Data are expressed as mean $\pm$ s.e.m. d and e,



Measurement of Ca_i^{2+} in a monolayer bilaterally perfused with Ca^{2+} -free Ringer, to which Tg and ATP were added sequentially to the serosal (S-Tg, S-ATP) or mucosal (M-Tg, M-ATP) perfusate as indicated in the trace ($n=3/2$ individuals each).

FIG. 3 Effects of serosal (S) and/or mucosal (M) extracellular Ca^{2+} on Ca^{2+} influx in HNE cell (field size, 30–40 cells) monolayers. **a**, A HNE cell monolayer is bilaterally perfused in nominally Ca^{2+} -free ($<10 \mu\text{M}$) NaCl Ringer solutions before adding ATP (100 μM) to the serosal bath. Ca^{2+} (1.3 mM) is added to mucosal (M- Ca^{2+}) and serosal (S- Ca^{2+}) bathing media as indicated in the trace ($n=6/3$ individuals). **b**, The protocol is identical to that shown in **a**, except ATP and extracellular Ca^{2+} are added to the perfusate in the reverse order to that shown in **a** ($n=6/3$ individuals). For series **a** and **b**, following a 60 min exposure to ATP, the readdition of serosal or mucosal Ca^{2+} caused cytosolic Ca^{2+} to increase to steady-state levels of $225 \pm 21 \text{ nM}$ ($n=6/3$ individuals) and $150 \pm 13 \text{ nM}$ ($n=6/3$ individuals), respectively. **c**, A HNE cell monolayer is bilaterally perfused in Ca^{2+} -free Ringer before adding and removing ATP to the serosal bath at the times shown in the trace. At about 45 min following ATP removal, Ca^{2+} (1.3 mM) is added to mucosal (M- Ca^{2+}) and serosal (S- Ca^{2+}) bathing media as shown. **d**, An identical protocol to that shown in **c** was done in monolayers initially perfused in Ca^{2+} -free Ringer, except that ATP was added to and removed from mucosal perfusate, and Ca^{2+} was first added to the serosal and then mucosal bath. For the ATP pulse studies, readdition of serosal or mucosal Ca^{2+} generated steady-state plateau levels of $146 \pm 15 \text{ nM}$ ($n=4/2$ individuals) and $113 \pm 7 \text{ nM}$ ($n=4/2$ individuals), respectively. These serosal or mucosal values are significantly lower ($P<0.05$) than the corresponding plateau levels obtained in the presence of ATP. **e**, Time-dependent changes in summed $\text{Ins}(1,4,5)\text{P}_3$, $\text{Ins}(1,3,4)\text{P}_3$, and $\text{Ins}(1,3,4,5)\text{P}_4$ resulting from a brief exposure to serosal or mucosal ATP in the absence of Li^+ ($n=3$ individuals). At time = 5 min, ATP was hydrolysed by the addition of 2 U ml^{-1} apyrase. Further, although $\text{Ins}(1,4,5)\text{P}_3$ was detected early (30 s) in the assay, at 5 min of ATP exposure, $\text{Ins}(1,4,5)\text{P}_3$ had returned to baseline with

70% and 30% of the inositol phosphates eluting as $\text{Ins}(1,3,4)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$, respectively (not shown), indicating that $\text{Ins}(1,4,5)\text{P}_3$ is a short-lived, rapidly metabolized species as has been documented in other cell systems^{15,16}. All data are mean $\pm$ s.e.m.

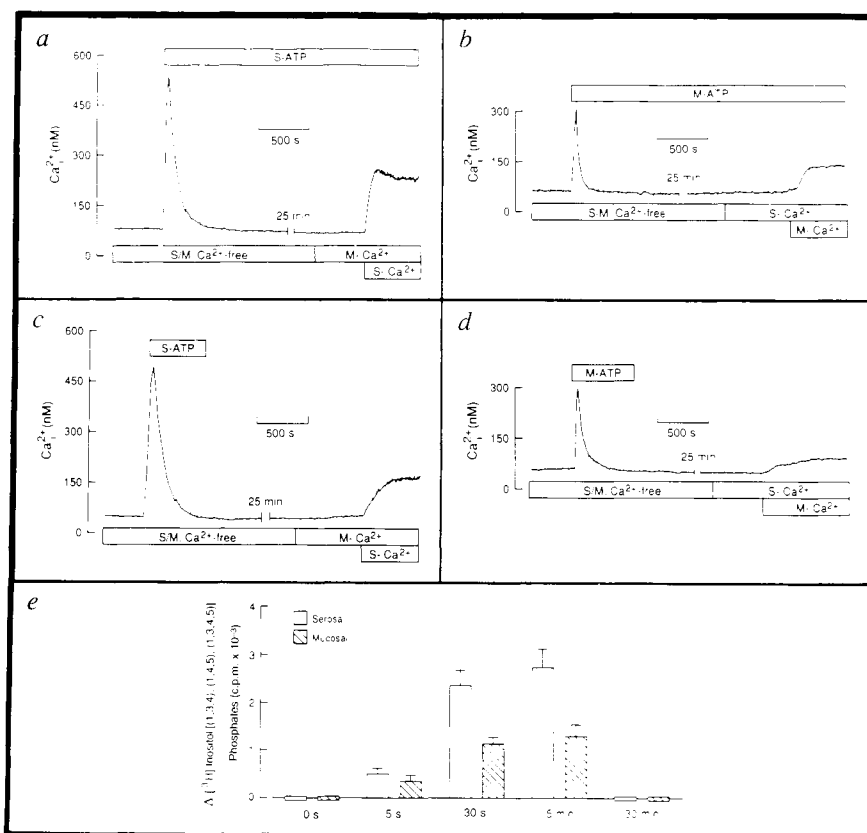
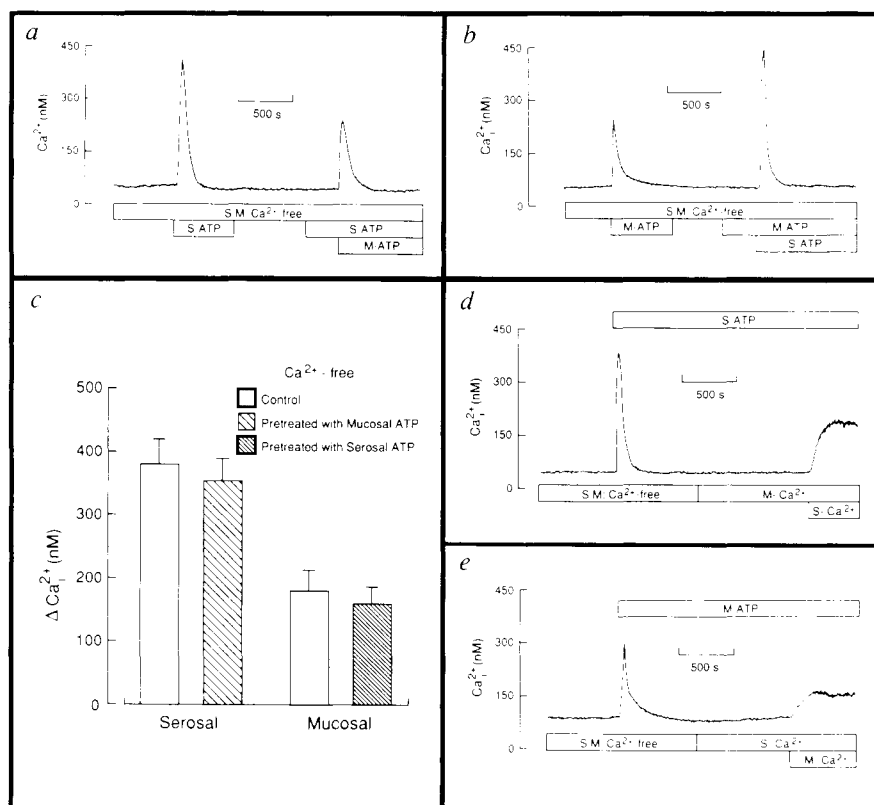


FIG. 4 Effects of serosal (S) and/or mucosal (M) ATP (100 μM) on intracellular Ca^{2+} release and Ca^{2+} influx in single cells within monolayers. For these studies, an image-plan pinhole (corresponding to a spot diameter of 3–5 μm) collected Fura-2 fluorescence arising from a single cell. Intracellular-free $\text{Ca}^{2+} = \text{Ca}_i^{2+}$. **a**, In nominally Ca^{2+} -free Ringer, extracellular ATP was applied sequentially to the serosal (S-ATP) and mucosal (M-ATP) perfusate as indicated in the trace. **b**, The protocol is identical to that shown in **a** except ATP is sequentially added to the mucosal and then serosal perfusates. Basal Ca_i^{2+} were similar in monolayers bilaterally perfused with NaCl Ringer containing Ca^{2+} (1.3 mM, not shown) or Ca^{2+} -free solutions: $73 \pm 8 \text{ nM}$ ($n=16/2$ individuals) and $64 \pm 10 \text{ nM}$ ($n=16/2$ individuals), respectively ($P>0.05$, Student's *t*-test). **c**, Summarized data showing the transient change of Ca_i^{2+} (ΔCa_i^{2+} , peak-basal) in naive (control; serosal $n=8/2$ individuals, mucosal $n=8/2$ individuals) cells within monolayers and in monolayers pretreated with serosal ($n=4/2$ individuals) or mucosal ($n=4/2$ individuals) ATP before activation of the P_2 receptors localized on the contralateral membrane. **d**, A monolayer is bilaterally perfused in nominally Ca^{2+} -free NaCl Ringer solutions before adding ATP to the serosal bath (S-ATP). Ca^{2+} (1.3 mM) is added to the mucosal (M- Ca^{2+}) and serosal (S- Ca^{2+}) bathing media as indicated in the trace. **e**, The protocol is identical to trace **d**, except ATP and extracellular Ca^{2+} are added to the perfusate in the reverse order. For series **d** and **e**, readdition of serosal or mucosal Ca^{2+} generated steady-state plateau levels of $192 \pm 16 \text{ nM}$ ($n=4/2$ individuals) and $132 \pm 10 \text{ nM}$ ($n=4/2$ individuals), respectively. All data are mean $\pm$ s.e.m.



actions persist ipsilateral to the stimulated membrane for up to 60 min but the factor does not act at the contralateral membrane distant from the internal stores emptied by receptor stimulation. Our data indicate that this soluble factor is not $\text{Ins}(1,3,4,5)\text{P}_4$, but do not distinguish between direct physical interactions between the plasma membrane influx path(s) and the Ca^{2+} stores/receptors⁸ or a soluble factor that is confined/metabolized within the local domain of the stimulated membrane.

Our studies revealed that activation of P_2 receptors regulates intracellular Ca^{2+} release and influx responses that are functionally confined to one surface of polarized epithelia. Identical responses are observed in single cells within a polarized monolayer as were obtained from fields (30–40) of epithelial cells, indicating that membrane-restricted regulation of internal Ca^{2+} release and influx is an inherent property of each individual cell within the epithelium (Fig. 4a–e). Thus, for internal Ca^{2+} release, it appears that $\text{Ins}(1,4,5)\text{P}_3$ generation and catabolism are colocalized to a cellular domain of the stimulated membrane. Activation of the Ca^{2+} influx pathway is also confined to the membrane of receptor activation, implying a topographically close relationship between the emptied state of the Ca^{2+} store and the influx path. We do not know whether the membrane-confined regulation of releasable Ca^{2+} stores and Ca^{2+} influx described herein is a general phenomenon in all epithelia^{9,10}, and further studies in other epithelia will be required to test this issue. However, this capacity appears critically important for airway epithelia, which are exposed to quite different environ-

ments. Sided, or membrane-specific, regulation of these cellular responses would thus give airway epithelia an ability to respond selectively to, for example, its airway luminal environment without perturbing the cellular homeostatic functions associated with basolateral membrane regulation. □

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Induction of the growth inhibitor IGF-binding protein 3 by p53

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TRANSCRIPTIONAL activation of target genes represents an important component of the tumour-suppressor function of p53 and provides a functional link between p53 and various growth-regulatory processes, including cell cycle progression (*p21/WAF1*)^{1–3}, DNA repair (*GADD45*)⁴ and apoptosis (*bax*)⁵. Here we use a differential cloning approach to identify the gene encoding insulin-like growth factor binding protein 3 (IGF-BP3) as a novel p53-regulated target gene. Induction of *IGF-BP3* gene expression by wild-type but not mutant p53 is associated with enhanced secretion of an active form of IGF-BP3 capable of inhibiting mitogenic signalling by the insulin-like growth factor IGF-1. Our results indicate that IGF-BP3 may link p53 to potential novel autocrine/paracrine signalling pathways and to processes regulated by or dependent on IGF(s), such as cellular growth, transformation and survival.

EB1 colon carcinoma cells, which carry an inducible wild-type p53 transgene under the control of a metallothionein promoter and undergo apoptosis upon induction of p53 (ref. 6), served as a model system to find new p53-induced target genes. Using a subtractive complementary DNA cloning approach⁷, we identified a number of enriched cDNA fragments derived from distinct p53-induced transcripts. Nucleotide-sequence analysis determined that one particular cDNA fragment was identical to a

region in the *IGF-BP3* gene (Fig. 1a)⁸. As shown in Fig. 1b, activation of the wild-type p53 transgene in EB1 cells by CdCl_2 stimulation leads to a pronounced accumulation of IGF-BP3 messenger RNA. This induction (~14-fold) is specific to the clonal p53-expressing EB1 cells and comparable to that of other previously characterized p53-response genes (for example, *p21/WAF1*, *A28* and *A26*)⁷. Consistent with these findings, IGF-BP3 mRNA expression is induced only at the permissive temperature in Saos-2-D4H cells expressing a temperature-sensitive p53 mutant, p53V143A (ref. 7; Fig. 1c).

Examination of *IGF-BP3* genomic sequences revealed potential p53-binding sites in the first (box A) and second (box B) introns, respectively (Figs 1a and 2a). These diverge by 2 or 3 nucleotides from the consensus p53-binding site RRRC-(A/T)(A/T)GYYY⁹. Electrophoretic mobility shift analysis (EMSA) using purified recombinant human p53 protein¹⁰ and anti-p53 monoclonal antibodies confirms that these DNA elements are specific p53-binding sites: a wild-type, but not mutant, consensus oligonucleotide competes for p53 binding (Fig. 2b). The weaker binding of p53 to the box-B oligonucleotide compared with the box-A oligonucleotide is consistent with its poorer similarity to the consensus p53-binding sequence. Both box A and box B sequences confer inducibility specifically by wild-type p53 in *cis* to a heterologous promoter when introduced into human Saos-2 cells (Fig. 2c), confirming the nature of these DNA sequences as active p53-responsive elements. Consistent with these DNA-binding studies, box A confers stronger inducibility by p53 than does box B. However, the synergistic response observed with 2 copies of box-B DNA raises the possibility that it may cooperate with box A in mediating p53 induction. In conjunction with the IGF-BP3 mRNA expression studies, these results indicate that the *IGF-BP3* gene is a candidate target for p53.

Consistent with a role for IGF-BP3 in p53 signalling, induced EB1 cells accumulate large amounts of IGF-BP3 protein in the culture medium (~4 nM; Fig. 3a). Two forms of IGF-BP3 (*M*_r 40K and 44K) corresponding to forms that inhibit cell growth^{11,12}, were detected. Immunodepletion experiments confirmed that the secreted IGF-BP3 is biologically active and inhib-

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its growth. Figure 3b shows that Saos-2 cells, which also express IGF-BP3 in response to p53 (Fig. 1c), are sensitive to the mitogenic effects of IGF-1 (1 nM) and that recombinant IGF-BP3 inhibits such activity in a dose-dependent manner (0–10 nM). Similarly, stimulation of DNA synthesis by IGF-1 is repressed

by the EB1 but not the control EB-conditioned medium (CM) (~50% inhibition). As shown by semi-quantitative western blot analysis, the concentration of IGF-BP3 in EB1-CM is ~4 nM, and thus consistent with a partial inhibition of IGF-1 when compared to the activity of purified recombinant IGF-BP3

FIG. 3 p53 induces the secretion of growth-inhibitory forms of IGF-BP3. **a**, EB1 cells secrete IGF-BP3 proteins in response to p53 expression. Parental EB and clonal EB1 cells were grown for 24 h in defined Hams F12 medium with BSA, with (+) or without (–) 6 μ M CdCl₂. Proteins secreted into the culture medium were analysed by western blotting. Detection of recombinant (r)IGF-BP3 protein standard is also shown. **b**, Saos-2 cells are sensitive to the mitogenic activity of IGF-1: inhibition by IGF-BP3. Saos-2 cells maintained in defined medium were treated with IGF-1 (1 nM) in the presence or absence of recombinant human IGF-BP3 (0–10 nM), or IGF-BP3 alone (10 nM), and effects on DNA synthesis determined by ³H-thymidine incorporation. Average counts $\pm$ standard error from triplicate cultures are shown. **c**, p53-induced IGF-BP3 secreted from EB1 cells inhibits IGF-1 mitogenic activity. Recombinant IGF-1 (1 nM) was added to control medium or conditioned medium from CdCl₂-stimulated parental EB cell and clonal EB1 cells, and tested for its ability to induce DNA synthesis in Saos-2 cells. Where indicated, conditioned medium was immunodepleted for IGF-BP3 using an IGF-BP3 antibody (aBP3), or 'mock-depleted' using a control anti-MDM2 antibody. Results from triplicate cultures are expressed as per cent of thymidine incorporation relative to control Saos-2 cultures treated with control medium (conditioned medium from untreated EB1 cells) supplemented with 1 nM IGF-1 (100%) or 1 nM IGF-1 + 10 nM rIGF-BP3 (0%). **METHODS.** **a**, Western blot analysis. Medium (2 ml) was concentrated by precipitation with 10% trichloroacetic acid (TCA) and analysed together with standards (rIGF-BP3) by western blot using a polyclonal or monoclonal antibody directed against human IGF-BP3, as recommended by the supplier (Accurate Scientific). **b**, Saos-2 cell DNA synthesis assay. Subconfluent cultures of human Saos-2 osteosarcoma cells growing in enriched medium (McCoy's medium supplemented with 15% fetal calf serum) were changed to serum-free Hams F12 medium supplemented with 0.1% BSA (crystalline, GIBCO BRL), with or without recombinant IGF-1 (1 nM; UBI) and increasing amounts of recombinant IGF-BP3 (0–10 nM; UBI), as indicated. After incubation, cells were pulsed by addition of ³H-thymidine (2 μ Ci ml⁻¹) for 3 h. Cells were washed in PBS, pH 7.4, and ³H-thymidine incorporation into acid insoluble material quantified by liquid scintillation counting. **c**, Immuno-depletion analysis. Conditioned medium from CdCl₂-treated EB or EB1 cells was dialysed against Hams F12 to remove metal ions. A sample of EB1 CM was then treated with anti-IGF-BP3 antibody or control antibody (aMDM2; Oncogene Science), and cleared using protein A-Sepharose. IGF-1 (1 nM) was added to the filter-sterilized conditioned-medium samples and analysed for its ability to induce DNA synthesis, as described for **b**.

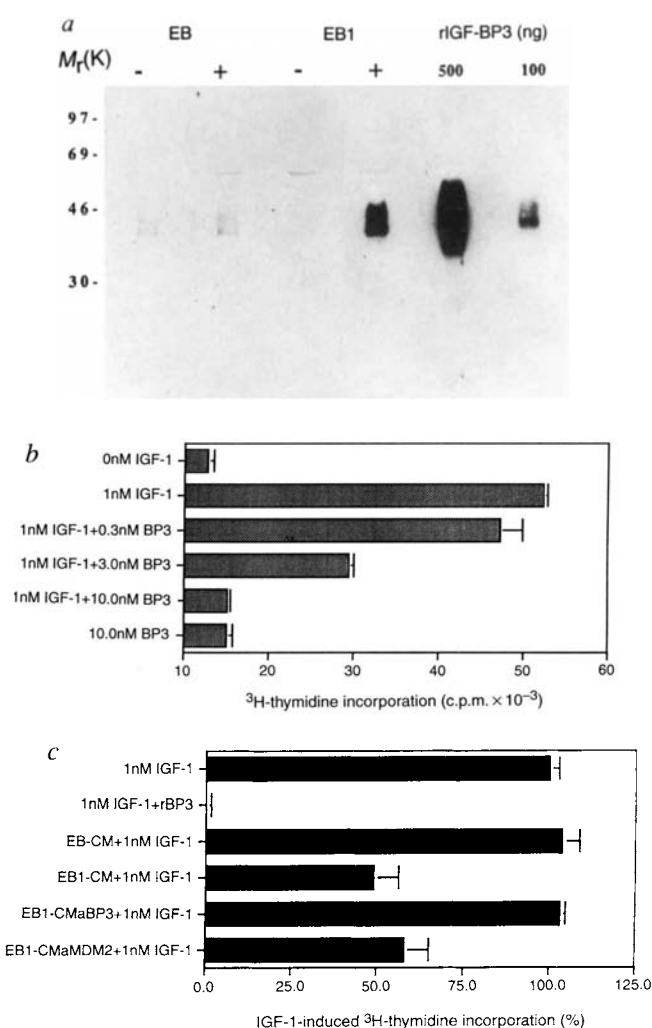
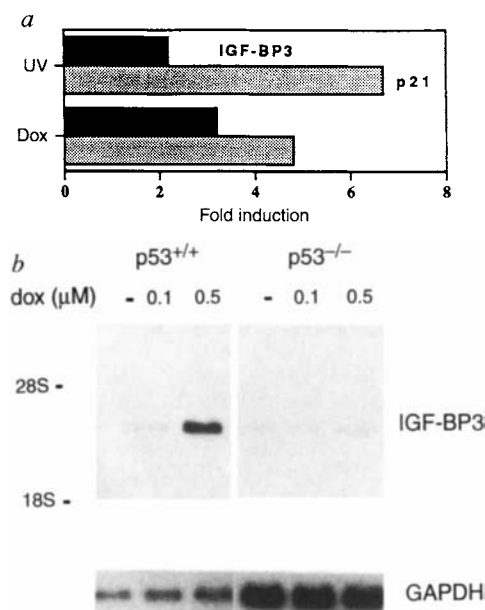


FIG. 4 p53-dependent induction of IGF-BP3 transcript levels in response to genotoxic stress. **a**, Induction of IGF-BP3 mRNA by DNA-damaging agents. Mid-log-phase cultures of normal diploid human fibroblasts (MRC-5, ATCC) were treated with doxorubicin (dox, 0.1 μ M) or were exposed to shortwave ultraviolet radiation (50 J m⁻²). After incubation for 20 h, total RNA was prepared and analysed by northern blot using a human coding region or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe and reduced-stringency hybridization conditions. Autoradiograms were quantified by densitometric scanning and normalized to the GAPDH signal. Fold induction relative to an untreated control is indicated. **b**, Induction of IGF-BP3 by DNA-damaging agents is dependent on p53. Primary embryo fibroblasts (MEFs) derived from p53 (+/+) or p53 (-/-) mice²¹ were treated with doxorubicin or UV radiation as indicated. RNA was prepared 20 h post-stimulation and analysed by northern blot analysis as described for Fig. 1. Ribosomal RNA marker positions are shown on the left.



(~50% inhibition at 3 nM rIGF-BP3). Indeed, specific immunodepletion of IGF-BP3 from EB1-CM results in full recovery of the mitogenic activity of added recombinant IGF-I (Fig. 3c), showing that p53 induces the synthesis and secretion of biologically active IGF-BP3 which presumably inhibits IGF-I by complexing with it.

As IGF-BP3 has a high affinity for IGFs and interferes with IGF signalling, it has been suggested that it is an autocrine/paracrine growth regulator¹¹⁻¹³, but the extent to which IGF-BP3 contributes to p53-regulated cell growth is not clear. Assessment of a potential autocrine versus paracrine (Fig. 3) role is limited by the nature of the p53-inducible cell lines available: for instance, EB1 cells are not protected from p53-induced apoptosis by excess IGF-I. This result may be interpreted in various ways. For example, it may be that IGF-BP3 and IGF-I are not important regulators of this process in EB1 cells (receptor levels are very low in these cells; S.V. and N.K., unpublished results); and/or it could be a result of p53 overexpression, causing overstimulation of cell-death pathways (*bax* is strongly induced in EB1 cells, for example; S.V. and N.K., data not shown); and/or IGF-BP3 may have effects that are independent of IGF. Consistent with this, IGF-BP3 inhibits the growth of fibroblasts in the absence of an IGF-I receptor¹¹; or IGF-BP3 could have a greater effect on cell-cell contact-regulated growth processes¹¹ *in vivo*. Down regulation of IGF-I or its receptor has little effect on the growth of tumour cells *in vitro* but triggers a massive apoptotic response *in vivo*^{14,15}.

A role for IGF-BP3 in mediating a cellular response to p53 is consistent with the induction of *IGF-BP3* gene expression in response to genotoxic stress. Treatment of subconfluent normal diploid human fibroblasts with the anticancer agent doxorubicin or with ultraviolet light induces *IGF-BP3* mRNA expression (~3.2- and 2.0-fold, respectively; Fig. 4a: the relative degree of induction may vary with growth conditions and associated changes in basal IGF-BP3 mRNA expression), although not as strongly as *p21/WAF1* mRNA expression. Doxorubicin induces *IGF-BP3* mRNA expression in cultured subconfluent mouse primary embryo fibroblasts (MEFs) expressing wild-type p53 (*p53*^{+/+} in Fig. 4b; ~10-fold induction) but not in MEFs with targeted disruption of the p53 gene (*p53*^{-/-}, same litter as *p53*^{+/+} mice; Fig. 4b). Thus, cellular p53 induces *IGF-BP3* gene expression in fibroblasts in response to DNA damage, but whether this represents a more widespread cellular response is not clear.

In summary, IGF-BP3 is probably a mediator in p53 signalling. It has been implicated in growth suppression and as a potential collaborator with p53 in regulating processes associated with senescence^{16,21}, as suggested for other binding proteins¹⁷, in IGF-independent growth responses¹¹, and in IGF-related mechanisms controlling cellular transformation and survival^{14,15}. Expression of prostate specific antigen, an IGF-BP3-specific and inactivating protease¹⁸, in human tumours¹⁸⁻²⁰ may be important for sensitization of some tumour cells to the actions of IGFs. A link between p53 and IGF-BP3 supports a potential role for this IGF-binding protein in p53-mediated cellular growth control. □

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Crystal structure of double-stranded DNA containing the major adduct of the anticancer drug cisplatin

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THE success of cisplatin in cancer chemotherapy derives from its ability to crosslink DNA and alter the structure. Most cisplatin-DNA adducts are intrastrand d(GpG) and d(ApG) crosslinks¹, which unwind and bend the duplex to facilitate the binding of proteins that contain one or more high-mobility-group (HMG) domains². When HMG-domain proteins such as HMG1, IXR (intrastrand-crosslink recognition) protein from yeast, or human upstream-binding factor (hUBF) bind cisplatin intrastrand crosslinks, they can be diverted from their natural binding sites on the genome and shield the adducts from excision repair³⁻⁵. These activities sensitize cells to cisplatin and contribute to its cytotoxic properties. Crystallographic information about the structure of cisplatin-DNA adducts has been limited to short single-stranded deoxyoligonucleotides such as *cis*-[Pt(NH₃)₂{d(pGpG)}]⁶⁻⁸. Here we describe the X-ray structure at 2.6 Å resolution of a double-stranded DNA dodecamer containing this adduct. Our information provides, to our knowledge, the first crystallographic look at a platinated DNA duplex and should help the design of new platinum and other metal crosslinking antitumour drug candidates. Moreover, the structure reveals a unique fusion of A- and B-type DNA segments that could be of more general importance.

A site-specifically modified, double-stranded deoxyoligonucleotide with the sequence d(CCTCTG*G*TCTCC)-d(GGAGACCAGAGG), where G*G* represents the *cis*-[Pt(NH₃)₂-{d(GpG)-N7(G₆)-N7(G₇)}] intrastrand crosslink, was prepared and crystallized. The structure was solved by conventional multiple isomorphous replacement (MIR) methods with three brominated derivatives (Table 1), one of which (T₃ replaced by U₃^{Bz}) diffracted to 2.6 Å and provides the basis for the following discussion. The crystals are triclinic with two molecules in the unit cell, affording two crystallographically independent views of the molecular structure. Within error, the results for the two duplexes are identical, the r.m.s. difference for all atoms being 0.4 Å.

The platinated DNA duplex dodecamers are significantly bent (Fig. 1a). The *cis*-[Pt(NH₃)₂{d(GpG)-N7(G₆)-N7(G₇)}] intrastrand crosslink distorts the DNA before crystallization⁹⁻¹¹, and the resulting bent structure must be accommodated by the crystal lattice. This is accomplished through three kinds of packing interactions between adjacent molecules (Fig. 1b). First, the two duplexes that make up the asymmetric unit are positioned backbone-to-backbone. This hydrophilic interaction is probably

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FIG. 1 *a*, Stereo image of one duplex of $\text{d}(\text{CCTCTG}^*\text{G}^*\text{TCTCC})\text{-d}(\text{GGAGACCAGAGG})$ where the $\text{-G}^*\text{G}^*\text{-}$ site has been modified by cisplatin. *b*, Packing diagram illustrating the interactions between adjacent molecules in the crystal lattice. One crystallographically independent molecule is drawn in white and the other in grey. *c*, Stereo image of the $\text{-G}^*\text{G}^*\text{-}$ platination site. The base pairs are propeller twisted but retain their hydrogen bonds. One of the amines and a phosphate oxygen of G_6 are within hydrogen bonding distance, 3.1 Å.

stabilized by divalent cations, but we have not been able to identify them. Second, one end of each duplex stacks on top of a molecule in an adjacent unit cell, a feature that occurs in many DNA crystal structures¹². Third, the other end of the duplex resides in the minor groove across from the cisplatin binding site of yet a third molecule. Such an interaction has been previously encountered in crystal structures of A-DNA¹³.

Coordination of the $\text{cis-}\{\text{Pt}(\text{NH}_3)_2\}^{2+}$ fragment to G_6 and G_7 bends the duplex toward the major groove without disrupting Watson-Crick hydrogen bonding (Fig. 1*a*). This result is consistent with an early NMR structural analysis of a cisplatin-modified duplex decamer, $\text{cis-}[\text{Pt}(\text{NH}_3)_2\{\text{d}(\text{TCTCG}^*\text{G}^*\text{TCTC})\cdot\text{d}(\text{GAGACCGAGA})\}]^{14}$. The central base pairs of the cisplatin-modified duplex, T5-A20, G6-C19, G7-C18 and T8-A17, are propeller twisted by -8 , -19 , -16 and -37 degrees, respectively, but remain hydrogen bonded (Fig. 1*c*). Although at 2.6 Å resolution the sugar 'puckers' cannot be identified from the electron density maps, their conformations can be inferred from distances between adjacent phosphates and the C3'-C4' (δ) torsion angles. Such an analysis (Fig. 2*a, b*) reveals that the C3'-endo conformation predominates to the 5' side and beyond the platinum adduct and that the C2'-endo conformation occurs on the 3' side with the junction of the two sections occurring at base pair T8-A17. A switch from C2'-endo to C3'-endo sugar puckers has been observed in the $\text{cis-}[\text{Pt}(\text{NH}_3)_2\{\text{d}(\text{pGpG})\}]$ and $\text{cis-}[\text{Pt}(\text{NH}_3)_2\{\text{d}(\text{CpGpG})\}]$ crystal structures as well as in several NMR studies of duplex oligonucleotides in solution¹⁵, but an abrupt change from a duplex resembling B-DNA to one resembling A-DNA promulgated well beyond the platinum lesion was unexpected¹⁶ and may be influenced by crystal packing.

The minor groove widths are all 9.2–11.2 Å, closer to the 11.0 Å width of canonical A-DNA than the 5.7 Å width of B-DNA¹⁷. The A- and B-type segments indicated by the sugar puckers and the phosphate-phosphate distances, however, are especially obvious when the platinated DNA helix is viewed end-on. A projection of the helix onto base pair C1-G24 shows similarity to A-DNA whereas a view looking down the opposite end of the helix at base pair C12-G13 shows a structure closer to that of B-DNA (Fig. 2*c, d*). The A-like portion of the structure also packs in a manner characteristic of A-DNA crystals with the end of one duplex extensively contacting the minor groove of another molecule (Fig. 1*b*)¹⁸. The section of the helix resembling B-DNA engages in end-to-end stacking, a frequent motif in B-DNA crystals. The A/B-type hybrid in this cisplatin-modified double-stranded dodecamer is unique and may be a recognition element for cellular proteins. A similar suggestion was made for a DNA fragment containing the restriction sequence for *HpaII* and *MspI* which has an alternating A-DNA/B-DNA conformation¹³.

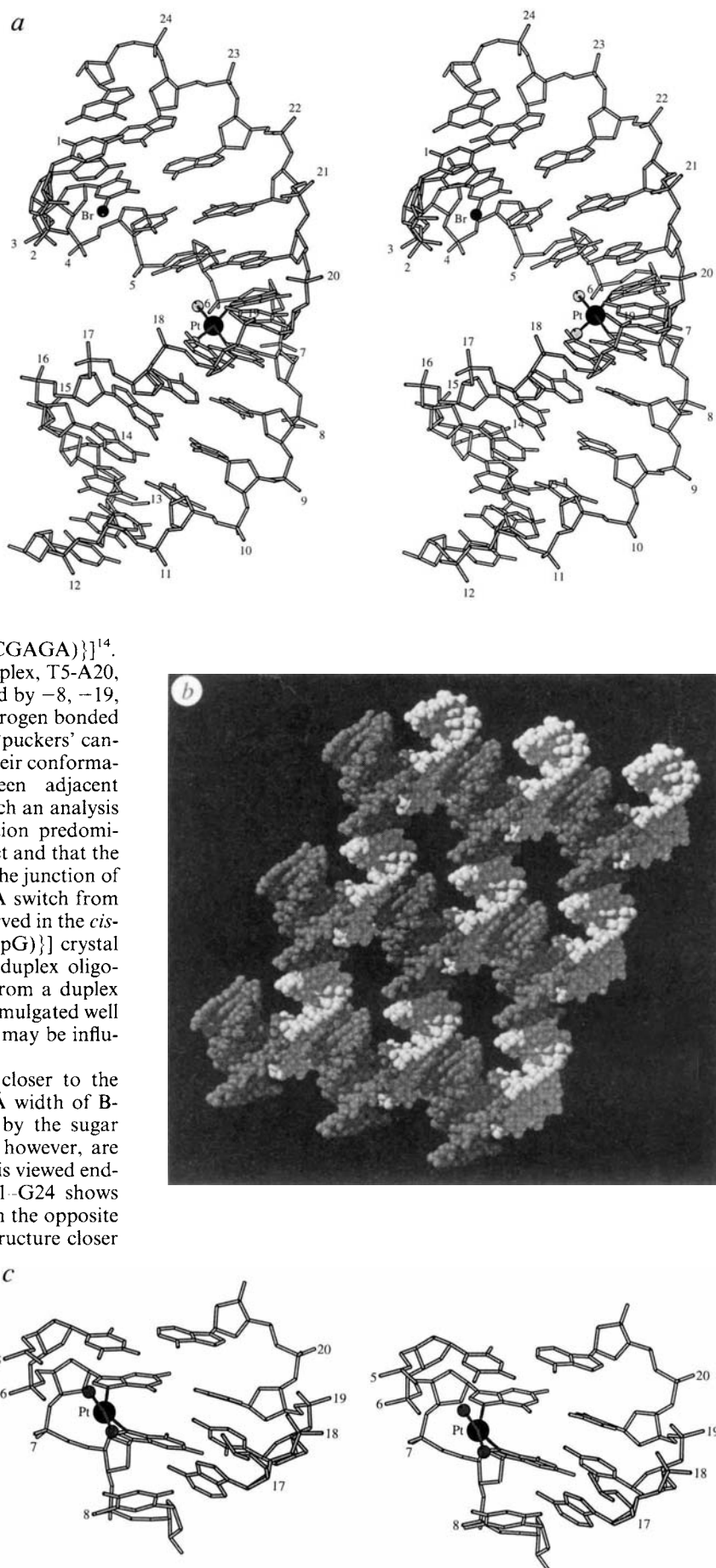


TABLE 1 Summary of crystallographic data

Crystal	Resolution (Å)	Unique reflections	Completeness (%)	R_{merge}^* (%)	Isomorphous difference (%)	Phasing power [†]
Native	3.0	3,793	97.7	6.2		
Br1	2.6	5,797	97.0	6.2	13.4	1.4
Br2	3.0	3,380	86.8	8.7	16.1	0.9
Br3	3.0	3,488	89.9	7.5	17.1	0.9

Sequences used:

Native	d(CCTCTG*G*TCTCC)-d(GGAGACCAGAGG)
Br1	d(CCU ^{Br} CTG*G*TCTCC)-d(GGAGACCAGAGG)
Br2	d(CCTCTG*G*U ^{Br} CTCC)-d(GGAGACCAGAGG)
Br3	d(CCTCTG*G*TC ^{Br} TCC)-d(GGAGACCAGAGG)

Crystal preparation: DNA containing the *cis*-[Pt(NH₃)₂{d(GpG)-N7(G₆)-N7(G₇)}] intrastrand crosslink was prepared as described previously¹⁰. Platinated and unplatinated deoxyoligonucleotides were purified once by ion exchange high-performance liquid chromatography (HPLC) and then twice by reverse phase HPLC, and annealed to form double-stranded DNA. Crystals of the platinated duplex and its brominated derivatives were obtained by using 0.2 mM DNA, 52 mM cacodylic acid (sodium salt, pH 6.0), 15 mM MgCl₂, 6 mM [Co(NH₃)₆]Cl₃ and 3% 2-methyl-2,4-pentenediol (MPD) in sitting drops equilibrated against a reservoir of 5% MPD at 4 °C. Crystals were allowed to grow for 9–12 months before being used for data collection. Data collection: The space group and unit cell were determined by using a Rigaku four circle diffractometer with Cu K α radiation. The space group is P1 with unit cell dimensions $a=47.01$ Å, $b=35.46$ Å, $c=31.27$ Å, $\alpha=82.79^\circ$, $\beta=84.75^\circ$, $\gamma=79.81^\circ$. The unit cell contains two cisplatin–DNA duplexes. Data were collected on a Marresearch imaging plate detector with Cu K α radiation, processed and merged with the programs DENZO and SCALEPACK (written by Z. Otwinowski, University of Texas Southwest Medical Center), and further processed by using the CCP4 program suite²³. An anomalous difference Patterson map was calculated to obtain the relative coordinates of the Pt atoms. Data from each brominated derivative were used to prepare single isomorphous replacement (SIR) maps, from which possible bromine atom coordinates were obtained. All combinations of platinum and bromine coordinates were refined by using MLPHARE from the CCP4 suite. The correct coordinates were chosen by inspecting maps made with the resulting MIR phases. Model building and refinement: The program O was used for model building²⁴. Initially a model was built to fit the maps calculated with native F_{obs} to 3.0 Å. After several cycles of positional refinement with X-PLOR²⁵, the phases obtained from the model of the native DNA were applied to the Br1 derivative F_{obs} to 2.6 Å. Eighteen iterations of model building, positional refinement, and phase combination were done. After inclusion of 29 water molecules in the refinement, the current R -factor is 0.203 and the current free R -factor is 0.245. (For the free R -factor, 10% of the data were set aside before refinement²⁶.) The r.m.s. derivations from ideal distances and angles are 0.015 Å and 3.1° , respectively. The r.m.s. deviation of the temperature factors is 1.9 Å².

* $R_{\text{merge}} = \sum |I_{\text{obs}} - I_{\text{avg}}| / \sum I_{\text{obs}}$, where the summation is taken over all reflections.

† Phasing power is (r.m.s. ($|F_H|_{\text{calc}}$)) / (r.m.s. (ϵ)), where the numerator is the r.m.s. calculated heavy-atom structure factor and the denominator is the r.m.s. lack of closure error.

Geometric details at the cisplatin binding site are evident from Fig. 1c, and a $2|F_o| - |F_c|$ electron density map is presented in Fig. 3. In the final refinement cycles, the Pt–N7 distances to G₆ and G₇ refined to 2.0 Å. Coordination of platinum to these nucleotides destacks the bases and, as in the *cis*-[Pt(NH₃)₂{d(pGpG)}] crystal structure⁸, one of the ammine ligands on platinum forms a hydrogen bond with a terminal oxygen

atom of the G₆ 5'-phosphate group (Fig. 1c). The roll angle between the G₆ and G₇ guanine ring planes is $\sim 26^\circ$, considerably less than the $\sim 80^\circ$ value for *cis*-[Pt(NH₃)₂{d(pGpG)}]⁸. In addition, the platinum atom is displaced by 1.3 Å and 0.8 Å, respectively, from the G₆ and G₇ ring planes. This result suggests that embedding the *cis*-diammineplatinum(II) d(GpG) fragment in a DNA duplex distorts the platinum coordination geometry. Such

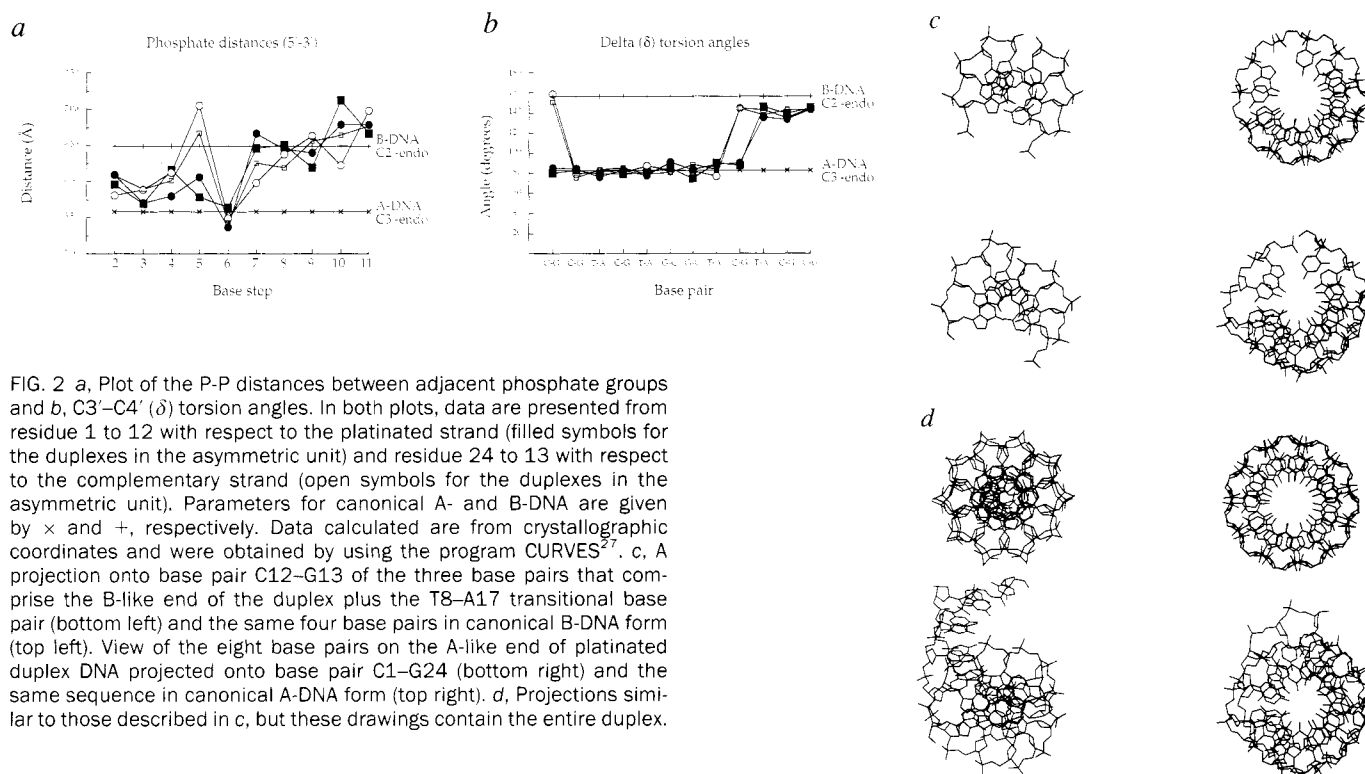
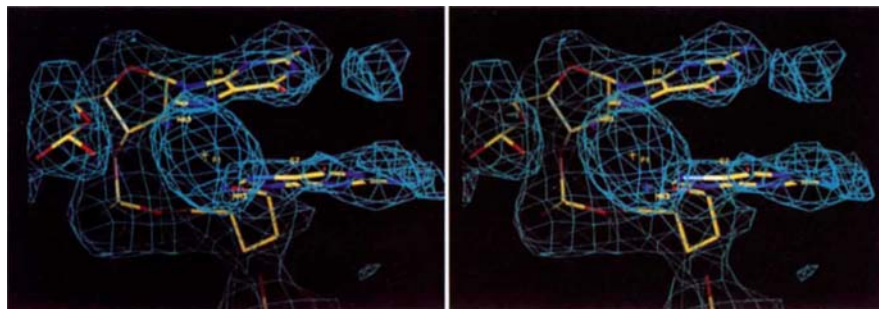


FIG. 2 a, Plot of the P-P distances between adjacent phosphate groups and b, C3'–C4' (δ) torsion angles. In both plots, data are presented from residue 1 to 12 with respect to the platinated strand (filled symbols for the duplexes in the asymmetric unit) and residue 24 to 13 with respect to the complementary strand (open symbols for the duplexes in the asymmetric unit). Parameters for canonical A- and B-DNA are given by $\times$ and $+$, respectively. Data calculated are from crystallographic coordinates and were obtained by using the program CURVES²⁷. c, A projection onto base pair C12–G13 of the three base pairs that comprise the B-like end of the duplex plus the T8–A17 transitional base pair (bottom left) and the same four base pairs in canonical B-DNA form (top left). View of the eight base pairs on the A-like end of platinated duplex DNA projected onto base pair C1–G24 (bottom right) and the same sequence in canonical A-DNA form (top right). d, Projections similar to those described in c, but these drawings contain the entire duplex.

FIG. 3 Stereo image of cisplatin bound to the d(GpG) site on duplex DNA. Shown in blue is the result of a $2|F_o|-|F_c|$ electron density map contoured at 1σ . The platinum coordination sphere consists of two amines and the N7 atoms of adjacent guanine residues.



a deformation at platinum afforded by the intrastrand d(GpG) crosslink is likely to be energetically costly. This energy might be released upon the binding of HMG domain proteins to *cis*-[Pt(NH₃)₂{d(pGpG)}] lesions in double-stranded DNA, which increases duplex bending to ~70–90° (ref. 19). Release of strain energy in this manner could increase the specificity of protein binding for the platination site.

Although the crystal structure clearly reveals that binding of cisplatin to the N7 atoms of adjacent guanosine residues bends the duplex in the vicinity of G₆ and G₇ (Fig. 1a), the angle could not be rigorously quantified. By using the three terminal base pairs in the B-type segment and the five terminal base pairs in the A-DNA segment, helical axes were calculated. One axis was translated such that its endpoint superimposed onto that of the other axis and, in this orientation, a ~39° bend was obtained for one duplex in the unit cell and a ~55° bend for the other. The difference reflects the lack of a point of intersection for the axes and the inability to determine an exact helical axis with only three base pairs in the B-type fragment. The bend in the crystal structure may be compared with the 34–40° value measured by gel electrophoresis for site-specifically platinated deoxyoligonucleotides^{9,11}.

The ability of cisplatin d(GpG), and presumably the closely related d(ApG)¹⁵, crosslinks to help stabilize an opened and flattened minor groove may be a key geometric feature that favours the binding of the HMG-domain proteins. It has been shown that when the HMG domain of SRY, the human testis-determining protein, binds to DNA, it partially intercalates an isoleucine into the minor groove²⁰. The resulting conformational distortion²¹ has geometric features in common with those induced by the *cis*-[Pt(NH₃)₂{d(GpG)}] intrastrand crosslink in the duplex investigated here and is intermediate between A- and B-DNA. Isoleucine intercalation occurs between two purine bases causing a roll of 19° towards the major groove. In both the SRY- and the cisplatin-DNA complexes, the major groove is compressed and the minor groove widened to resemble A-DNA. The resulting hydrophobic surface in the minor groove presents a target for interaction with the HMG domains of SRY and related proteins.

Whether or not packing interactions induce the present structure, the results suggest that the major d(GpG) and d(ApG) adducts of cisplatin prepare double-stranded DNA for the conformational switch required for HMG-domain protein binding. This property allows SRY and other HMG-domain containing proteins to recognize the major adducts of this anticancer drug in a structure-specific manner²². Moreover, because such an interaction may be critical for antitumour activity, it would be of interest to modify ligands on platinum to stabilize further the conformational changes induced by drug binding. □

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A metalloprotease-disintegrin participating in myoblast fusion

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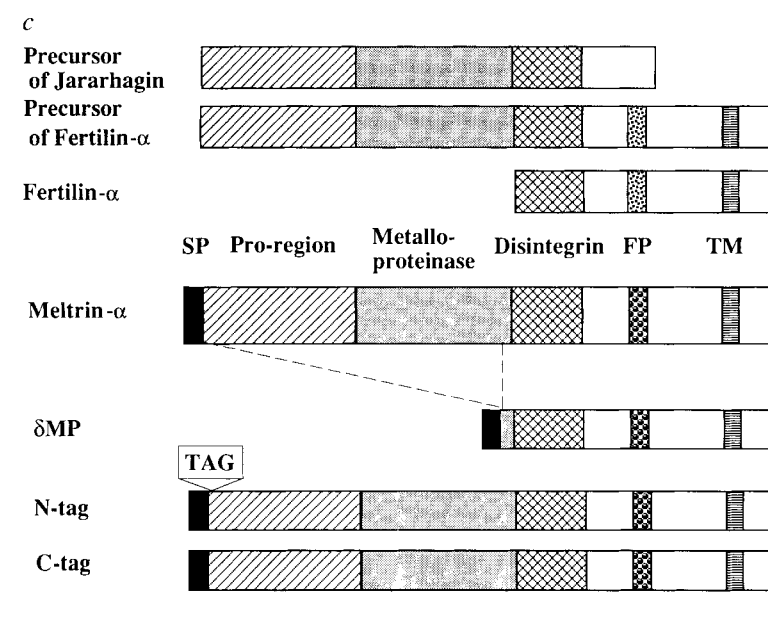
SKELETAL muscle development involves the formation of multi-nucleated myotubes. This is thought to proceed by the induction of differentiation (acquisition of fusion competence) of myoblast cells, their aggregation, and union of their plasma membranes^{1–3}. Various membrane proteins including N-cadherins^{4,5} and M-cadherins⁶, N-cadherins^{7–9} and V-CAMs¹⁰ and integrins^{10,11} participate in myotube formation, but the molecular mechanisms of muscle cell fusion are poorly understood. Here we report the identification of three new, myoblast-expressed gene products, meltrin- α , β and γ , with homology to both viper haemorrhagic factors^{12,13} and fertilin (PH-30)^{14,15}, a membrane protein involved in egg-sperm fusion. Meltrin- α , a member of the metalloproteinase/disintegrin protein

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FIG. 1 Primary structure of α , β and γ . Structures of meltrin- β and γ were deduced from DNA sequences of reverse transcription-polymerase chain reaction (RT-PCR) products. Nucleotide sequences reported in this study will appear in the GSDB, DDBJ, EMBL and NCBI databases with accession numbers D50410, D50411 and D50412. α , Comparison of polypeptide sequences of meltrin- α (Ma), β (Mb) and γ (Mg), with jararhagin¹³ (JR), MS2, which is a macrophage-specific antigen²⁶, and fertilin- α ¹⁵ (Fa). Similarities to EAPI²⁷ and MDC²⁸ are omitted. Potential sites for glycosylation and phosphorylation by protein kinase C are marked with asterisks and triangles, respectively. Also shown are Zn, a potential zinc-binding site; FP, fusion peptide-like sequence; and TM, a putative transmembrane region. b , Similarity between meltrin- α and the fusion peptide of Sendai virus, which is synthesized as a precursor and subsequently cleaved (shown by the arrow) to the biologically active form composed of F₁ and F₂. The fusion peptide (underlined) is positioned at the N terminus of F₁ (refs 16, 17). c , Schematic diagram of meltrin- α , related proteins, and constructs used in this study. δ MP is the construct in which the pro- and metalloproteinase regions are deleted to imitate fertilin- α . N-tag and C-tag are the constructs containing oligopeptide tags (SLASDVKDDDDKGT) at the N and C termini, respectively. All the constructs were expressed under control of a promoter of the elongation factor 1 α gene (pEFBOS)²². SP is a putative signal peptide. METHODS. The myogenic cell line used for the cDNA screening is the one derived from C3H10T1/2 and expresses myogenin inducibly (K.K. and A.F.-S., unpublished data). cDNAs were amplified by RT-PCR with degenerative primers for ED/ECDG (corresponding to 433-438) and KCGKLC (577-583). Similarity was found by searching the EMBL and GenBank databases.

Ma					MAERPAR	RAPARARLL	ALAGALLAPR	AARGMSLWDQ	RGAYEVARAS	LLSKDPGPGP	QSPAKDHEP	67			
MS2					CSRSHHAWP	LAARQLMDTS		SSPWTSEFAP	VKQYEVWVPR	RLAASRSRRR	LPSHWQVQYE	59			
JR										ATRP	KGAVQPKVE	14			
Fa					MRSGSMASV	RNTISFSASL	QKAHVVLHVA	RSLLQTCILL	MVNRILGLVP	GHLCVRLVTK	LLVETVLPH	100			
											INCHLGPVHY				
											SSYEIVIPES				
											LTVKGSQDGG				
Ma					VLTVQLQES	RDILISLERK	EGTIANGFTE	THYLDGTDY	SLRNHTDHC	YHGHVQGD	AGVSLSTCS	DLRGLIMFEN	KTSLDEPKRN	TDSYKILPA	167
MS2					SLSYALGTSG	HVFTTHRRKN	RDILGSSYTE	LYSAANGSEF	TEQLQEQDHC	INOGHVEEYE	GSAASISTGA	GLRGFFRVGS	TVHLTEPLDA	DEEGQHAMYQ	159
JR					AMQYEFKVG	EPVYHHLK	KSLFSKDYSE	THSPGKEI	TYTPFVEDHC	YHGHRIENDA	DSTASISACN	GLRGFFRVGS	ETVFTTEPL	PDSEAHAFK	114
Fa					GRISYMLTIQ	GHKCIHLKV	KRDYFVDDFP	VYSYHNNVR	QEPSTIARD	HDEYIEAS	SEFVSASAS	GLRGILIKEN	TSIGTEPLS	SQRFEHVLTY	200
Ma					ESMTNIGLC	SSOHNSSNLT	MEDVSPGTSQ	MEARRHKRET	LKMYFYVELV	IVADNREFQR	QSKOLEPKVK	RLIEIANHVD	KFYRPLNRE	VLGVGVVND	267
MS2					A--KHLQKA	STCGVDTNL	NDLGPRALAI	YEAQPRNLI	PREPVEVELY	VVADSCFPK	LG--SREAVR	RVLGVVNHVD	KLQELSPFV	VLVGLIANK	256
JR					YENVEKEDA	PKMCGVTQNW	KSYEPKAS	QLPFTAEQQR	YDPYKTEFF	VVVGQTVTK	NNGDLPFA	RYMELIIVN	EIERLYMHV	ALVGLHASN	214
Fa					MARQAPVSCR	ASAKDSQAVS	TSWQGSRRP	HSVQALSSYL	VVHTKYVEMF	VVVGQTVTK	WESVNETVY	RVVDILALAN	IFREGINTEV	VLGVGVVTE	300
Ma					IPKCSISQEP	FTRHEFLDA	RHKLLERKS	HDNAQLISGV	YFOCTTICMA	FVSMCTAEQ	SGGVVMDHSD	SPLGAAVTLA	HELGHNFKN	HDLLERGCSC	367
MS2					-DRFYISRYA	NVTENELSL	EQNLGGQHP	HDNVQLITGV	DFICSHVSLA	KVSALC-SRH	SGANVQHSK	NSIGVASTVA	HELGHNLGVS	HDIDIPGVC	354
JR					GDKITVKPVR	DYTLNLSABA	RKTDLLLRK	HDNAQLITAI	DENPGTICMA	YVSGMCHPKR	SVETLVQVSP	INLVAVVIMA	HEGHNLGTH	HDIT--GSQSC	312
Fa					GLLIEVVDL	RVTNRNRRK	RQKLLPVR	HDVAMHIVC	HHPESSSRA	FLNGASSSGF	AAAEAFHRE	DAVLSAALV	HELGHNLGTH	HDH--SAGVC	397
Ma					EMAAKGGCI	VNESTPEPPE	MVSSSSSRK	LEASLPGMG	MLENLPEV	KQAPGGRKC	NGYVEEGEC	DCEPEEPT	NPCCNATTC	LKPDVCAHG	465
Mb															
My															
MS2					PEPBEGGCI	VNESISKFP	RIFSRCSKT	LESFVKPQT	GLTNVPE-DV	NRFVGGPVCG	NLFVEHSEC	DCEPEEPT	NPCCNATTC	LKPDVCAHG	452
JR					GDYP---CI	VEITISNEFS	KFSNCSYIQ	CWDFIMNHNP	GLTINPE-LG	TDITSPVCG	NELLEVGECC	DCEPEEPT	NPCCNATTC	LKPDVCAHG	406
Fa					MDKH---SL	LOENITE--E	SGFSNCSYD	FYHFEHRE	ACLENPEHKK	ARRRRAATCG	NGVVESECC	DCEV--NCDT	SECC--QAN	LKPDVCAHG	489
Ma					QCCEDQQLKP	PGTACRGSN	SCDLPEECTG	TAPHCPANVY	LNDGHECOGV	DGYCYNGHQ	THEQCVTLW	GGAKPAPGI	CFERNVSAGI	FYNGCGKSK	565
Mb					SCCHQKVA	PCTQCEQVR	QCDLPEECTG	KSPHCPNIVY	QMDGTCPESG	QAYCYNGML	TYQBCOOLW	GGAKPAPGI	CFERNVSAGI	FYNGCGKSK	132
My					DCCQCKCFR	GSMSCKSTS	ECDLPEECTG	SSOFCHPDPF	IQNGVPCNS	KAYCYNGML	YDAGCOVIF	GGAKPAPGI	CFERNVSAGI	FYNGCGKSK	132
MS2					TCHEKVKVR	ASEVCRISK	KODLPEECTG	RKTPCEDAF	QNGTPCES	GYCYNGSC	FLAQCCDLW	GGAKPAPGI	CFERNVSAGI	FYNGCGKSK	546
JR					DCCQCKCFR	SSSTCPAEMS	ECDLPEECTG	QSSECPADVF	HKMGCELDN	YCYCYNGML	IMYHGVNCP	GADVVEADS	CF-KDNQKN	YCYCYNGML	504
Fa					LCCSCQVYN	SEYLECPVSG	PDLPEECTG	QSGCKPLDT	KQDGTCEG	-FFVSKGCT	DGIGQATVF	GGAKPAPGI	CFERNVSAGI	FYNGCGKSK	588
Ma					-DAFAKGLR	DAK--GKIQG	QSGARREVIG	TNAVSIETNI	QDQEGRIIL	RGTHVYVLE	MDPSLVLAG	TKCAEGRIEL	NRRQNI-SV	FGVHKCAMC	662
Mb					-GQYRKQSP	DAK--GKIQG									150
My					-BEYKCATG	NAL--GKIQG									151
MS2					-GRINR-GAL	YCEGGQPL-		-----ER	SFCTFSNHG	VCHALGTEN	IDTFELVLOS	TKCBEGHVM	DGSCODLRVY	RSENCASKN	626
JR					-GKIRPAPE	YVH--GRLYV		-----KDS	B---GQNP	KMFY---SND	DEHKEVLE	TKCADGVVS	NGHVDVATA	Y-----	571
Fa					PTTYVGS	SGD--GKILF	TESSLEPPR	-----ALFAA	QIPHDDWCW	SISNFGDPA	SETGANGAG	SCASGAGV	MAQSTFTLD	TANCASBEM	683
Ma					HGRVYCNRRK	NCHCBAAWAF	STDSGPIRQ	DNGLTVGIL	SLILCLLAG	FVYVYKRTL	MLLFTHKKT	TMEKLRVHE	SRTPSGPHLG		762
MS2					NH--SVGNHRR	ECHCRKCAF	STDSGPIRQ	STSLPVSVV	SLVILVAMV	IVAGIVYRK	APRQIQRSV	APKPS---			707
Fa					NENIICNLG	HCRGSDGFR	STDSGPIRQ	STPTAPPKPT	QTKASSEN	ALIC--ILVI	LLILVICAI	CLGIPAEAP	PPPEEEAGE		783
Ma					QAHHTPGKGL	LMNRAPHFNT	PKDRHSLKQ	NMDISRLDA	RAVPQLQSPQ	RVLLPLHQT	RAPSGPAPL	PASPAVRQAQ	GIRKSPSPK	PLADPLSRT	862
MS2					---GLSNPLF	YTRDSSL-PA	KNRPDPSET	VSTNQBRPI	AKPKRP---	-----PPAP	PGAVSSS-PL	GVVYAPKIP	NQFRDPPK	PLBELKPKQV	792
Fa					LEEEPEPEPE	PEEEAAEE	D								804
Ma					SRLTSALVRT	PGQEPHRE	APIRPAKQ	VPRFSNAYI	K						903
MS2					KPTFAPPTP	VKPGTGTV	GATQCGGPK	WALKVPIQK-	R						832

b Meltrin- α IQCGGASRPVIGTNAVSIETNIPOQEGRIILCR----- 581-614
Sendai virus GVPSRRFFGAVIGTIALGVATSA-QITAGIALAEAREAKRDIAL 111-153
↑



family, appears to be required for myotube formation. Involvement of a fertilin-related protein in myogenesis suggests that there are common mechanisms in gamete and myoblast fusion.

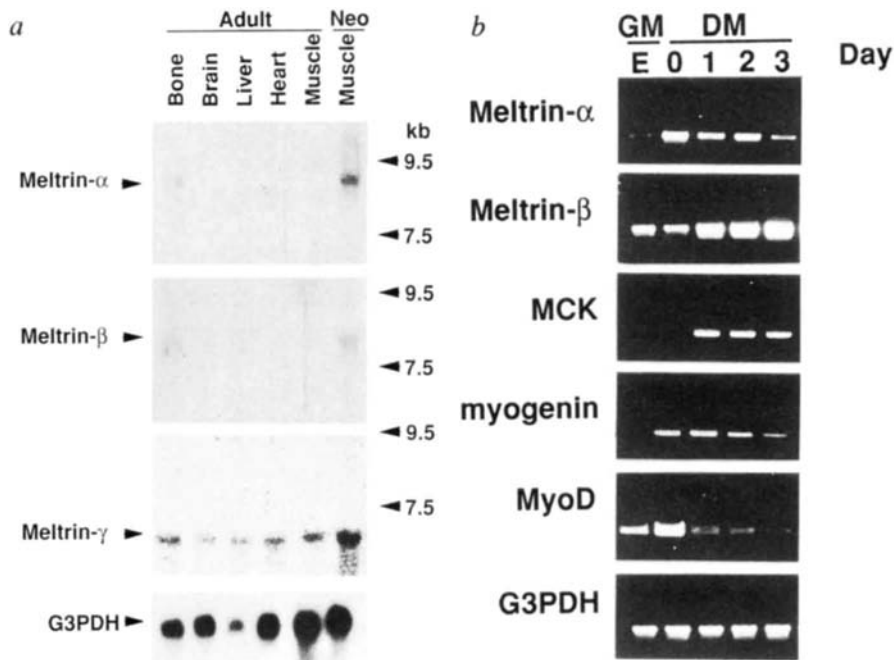
We have postulated that muscle cell and egg-sperm fusion events are dependent on fusion proteins, in a mechanism that is also used by viruses^{16,17}. Existence of a sequence similar to the potential fusion peptide of rubella virus in fertilin- α ¹⁵ led us

to attempt to isolate fertilin-related genes expressed in muscle. Complementary DNAs prepared from a mouse myogenic cell line were amplified using degenerative primers for conserved amino acids between fertilin- α and β , which allowed us to identify three new genes termed meltrin- α , β and γ (Fig. 1a). Northern blotting revealed specific expression of meltrin- α and β messenger RNAs in muscle at embryonic (data not shown) and

FIG. 2 Expression of meltrin- α , β and γ mRNA.

a, Northern blot analysis of meltrin- α , β and γ transcripts from various tissues of mouse. Note that the bone which contains multinucleated osteoclasts was the only tissue that expressed meltrin- α and β mRNAs (9.0 and 8.5 kilobases (kb) in length, respectively) in adults. In neonates, these mRNAs were expressed in muscle in addition to the bone (data not shown for the bone). G3PDH, glyceraldehyde 3-phosphate dehydrogenase. b, RT-PCR analysis to show that the meltrin- α gene is activated at initial stages of differentiation of C2 cells. mRNA was prepared from exponentially proliferating cells (E), cells at semi-confluence (O), and cells cultured in differentiation medium for 1, 2 and 3 days after semi-confluence (DM 1, 2 and 3). In proliferating cells, expression of meltrin- α mRNA was very low in contrast to moderate expression of meltrin- β mRNA. Note that transcripts of these genes were activated after differentiation in distinct manners; timing of activation of the meltrin- α gene was similar to that of the myogenin gene, a marker of initial stages of differentiation, and preceded activation of the meltrin- β gene and the onset of muscle creatine kinase gene, a marker of later stages of differentiation.

METHODS. For northern blot analysis, poly(A)⁺ RNA (5 μ g for each lane) was run on an agarose gel containing formaldehyde, transferred to a membrane (Hybond-N, Amersham), and hybridized with radiolabelled cDNAs (the region of disintegrin domain) for meltrin- α , β or γ . Total RNA of muscle or the bone was prepared from legs. C2 cells were induced to differentiate with



differentiation medium (DME medium containing 2% horse serum). Conditions of PCR were determined to provide quantitative information on each transcript. The primer sequences are available from the authors on request.

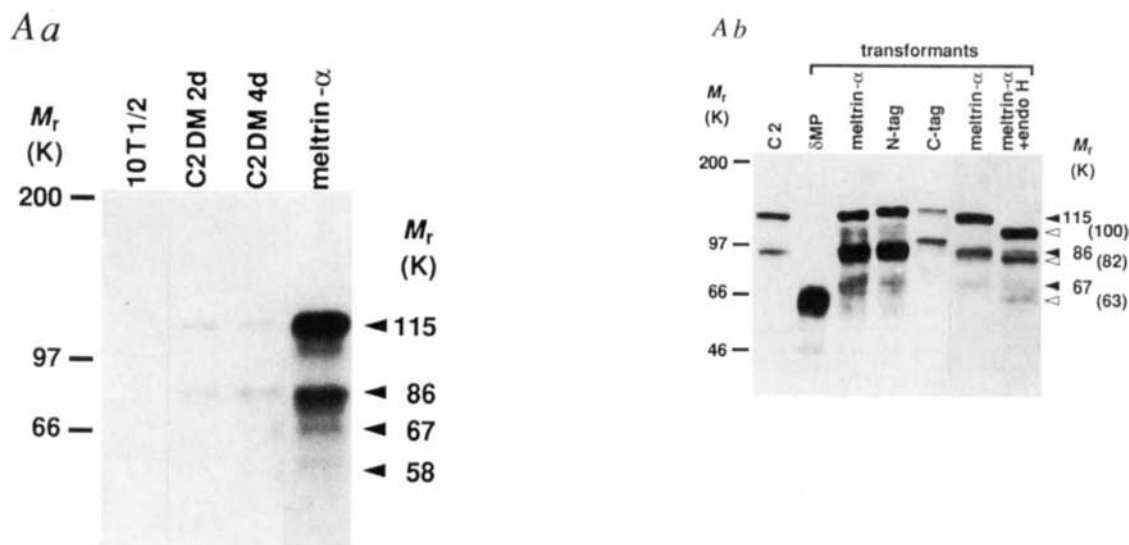


FIG. 3 Expression of meltrin- α in C2 cells. a, Immunoblot analysis with an anti-meltrin- α antibody (anti-Ma). a, Meltrin- α of different sizes expressed in C2 (differentiated for 2 and 4 days: DM2d and 4d) and in meltrin- α -transformants, but not in embryonic fibroblasts, C3H10T1/2 cells. Note that 67K and 58K proteins appeared after differentiation progressed (DM4d): low abundance could reflect their expression being restricted at the small, actively fusing interface. b, Translation products of transfected meltrin- α constructs; C2 cells were transfected with the

full-length (meltrin- α), δ MP, N-tag and C-tag (Fig. 1c). Expression of N-tag results in a slight increase in size of the 115K protein, whereas expression of C-tag resulted in shifts of all the species, suggesting that the 115K form undergoes proteolytic cleavages of N-terminal regions of the disintegrin domain. Retardation of the tagged proteins in electrophoresis may be partly due to the stretch of charged residues in tags. Right lysates of meltrin- α -transformants after digestion with endoglycosidase H. Sizes of the various forms before and after digestion are

neonatal stages, and in bone from embryo to adult, and showed ubiquitous expression of meltrin- γ mRNA (Fig. 2a). These three genes were also transcribed in a myoblast cell line, C2 (Fig. 2b). Meltrin- α mRNA in particular increased dramatically in response to the induction of differentiation in a similar fashion to the myogenin gene, a marker of initial stages of differentiation¹⁸, suggesting that meltrin- α is associated with early stages of myotube formation.

Meltrin- α cDNA encoded a transmembrane protein that belongs to a member of a metalloproteinase/disintegrin family (Fig. 1c). Furthermore, meltrin- α contains a sequence that is similar to the fusion peptides of paramyxoviruses, including Sendai virus^{16,17} (Fig. 1b), raising a possibility that meltrin- α has a fusogenic property.

An antiserum raised against the disintegrin domain of meltrin- α (anti-M α) detected proteins in C2 after differentiation (Fig. 3Aa); they also appeared as translation products of a meltrin- α transgene. Changes in size after insertion of peptide tags (carboxy and amino tags; Fig. 1c) and after the removal of sugar moieties indicate that meltrin- α is produced as a glycoprotein of relative molecular mass (M_r) 115K that is processed to a 86K form that lacks the pro-region and to smaller forms that lack the metalloproteinase domain (Fig. 3Ab). Venom disintegrins and fertilin undergo similar proteolysis^{19,21}.

Immunocytochemical staining of C2 with anti-M α and with an anti-myogenin antibody showed that meltrin- α was expressed in differentiation-induced cells, that is, myogenin-positive myocytes and myotubes (Fig. 3B). This led us to assume that meltrin- α functions after differentiation, perhaps during cell-cell or cell-matrix interactions of differentiation-induced cells and/or fusion itself.

To examine the roles of meltrin- α in myotube formation, the induction of meltrin- α in C2 cells was suppressed by expression

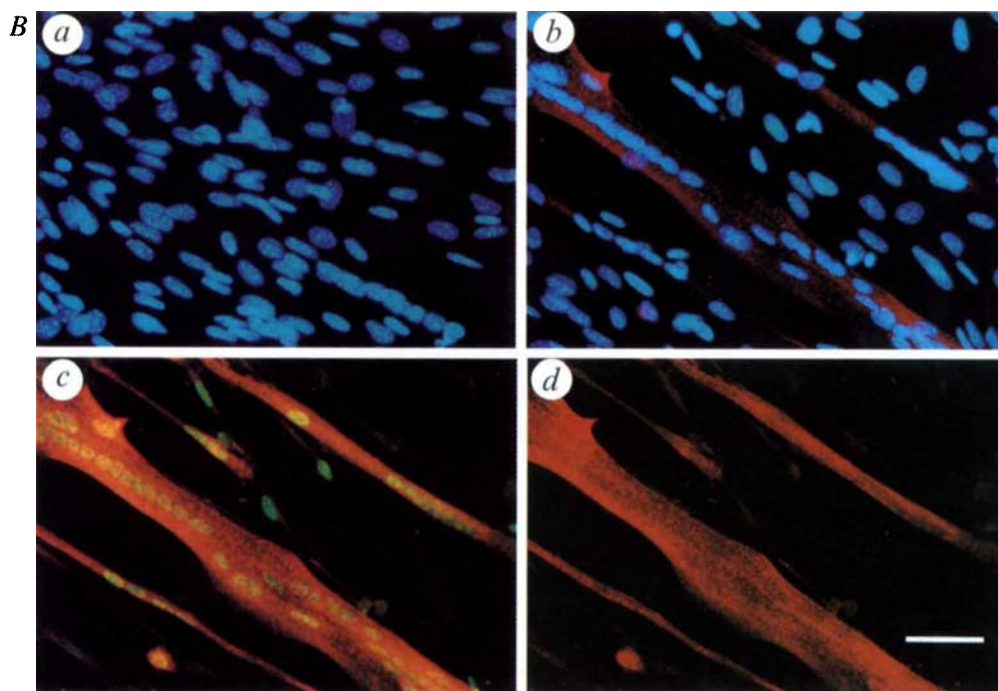
of the antisense RNA (AS; the antisense construct for δ MP in Fig. 1c) under the control of a strong promoter (pEF-BOS²²). As shown in Fig. 4A, myotube formation was suppressed in those stable AS-transformants that expressed a lower level of meltrin- α than the parental C2. The effect of AS was further confirmed by using primary colonies of individual transfectants to analyse the effect more statistically. The population of colonies with a high fusion index prominently decreased in the AS-transformants compared with the vector-transformants (Fig. 4B). These data strongly suggest that meltrin- α is essential for myotube formation.

We also investigated the effects of overexpression of meltrin- α in C2 cells. Based on the assumption that the processed 67K and/or 58K proteins represent active forms of meltrin- α , we also examined expression of a truncated construct (δ MP; Fig. 1c). As shown in Fig. 4, expression of δ MP facilitated fusion in the differentiation medium, whereas overexpression of full-length meltrin- α suppressed fusion. The opposite effects of full-length and truncated constructs suggest that proteolysis is important for regulation of meltrin- α function; the disintegrin and the following fusion peptide-like regions are probably responsible for enhancement of fusion, but the full-length meltrin- α might represent a cryptic form whose overexpression prevents more processed forms from facilitating fusion.

When cultured at higher density, the AS- and full-length-transformants fused more efficiently than before, whereas the cells differentiated at lower density tended to be round and often detached. In contrast, most of the δ MP-transformants exhibited disorganized aggregation and fusion to each other irrespective of their density (Fig. 4D). Furthermore, the myosin heavy-chain gene was activated similarly in these three types of transformants and the vector-transformants (data not shown). Taken together, these results suggest that meltrin- α is involved in cell-cell or cell-matrix interactions after differentiation rather than stimulating differentiation.

The expression of δ MP in L929 fibroblasts did not cause their aggregation or fusion (data not shown), indicating that meltrin- α is not a homotypically interacting adhesion molecule or a fusion protein. It is conceivable that meltrin- α exists as a heterodimer, as does fertilin^{14,15,21}, or that it requires other factors to express its function. Cumulative data on interactions of venom disintegrins and fertilin with platelet²³ and egg integrins²⁴, respectively, raise the possibility that the disintegrin domain of meltrin- α works as a ligand for integrins or other receptors expressed on the surface of differentiated cells.

Finally, several lines of evidence suggest that muscle cell fusion involves metalloproteinase-sensitive mechanisms^{3,25}. The metalloproteinase domain of meltrin- α could be one such proteinase that promotes cell-cell interactions by degrading some extracellular matrix, as is the case with venom haemorrhagic factors. The metalloproteinase-disintegrin family, which includes meltrins, may



indicated with filled and open triangles, respectively. B, C2 cells differentiated for 2 days were stained with preimmune serum (a) or anti-M α (b, c and d: rhodamine, red) and an anti-myogenin monoclonal antibody (c: FITC, green) after fixation with 4% formaldehyde. Nuclei were labelled with DAPI (a, b). b-d show the same field. The cells expressing meltrin- α are also positive for myogenin (yellowish). Scale bar, 50 μ m.

METHODS. A rabbit anti-M α was raised against a chimaeric protein of glutathione S-transferase (GST)²⁹ and the disintegrin and following cysteine-rich regions of meltrin- α (amino acids 483-636), and was affinity purified. Fusion of C2 cells was not blocked with anti-M α (data not shown). Immunoblots were detected using ECL (Amersham).

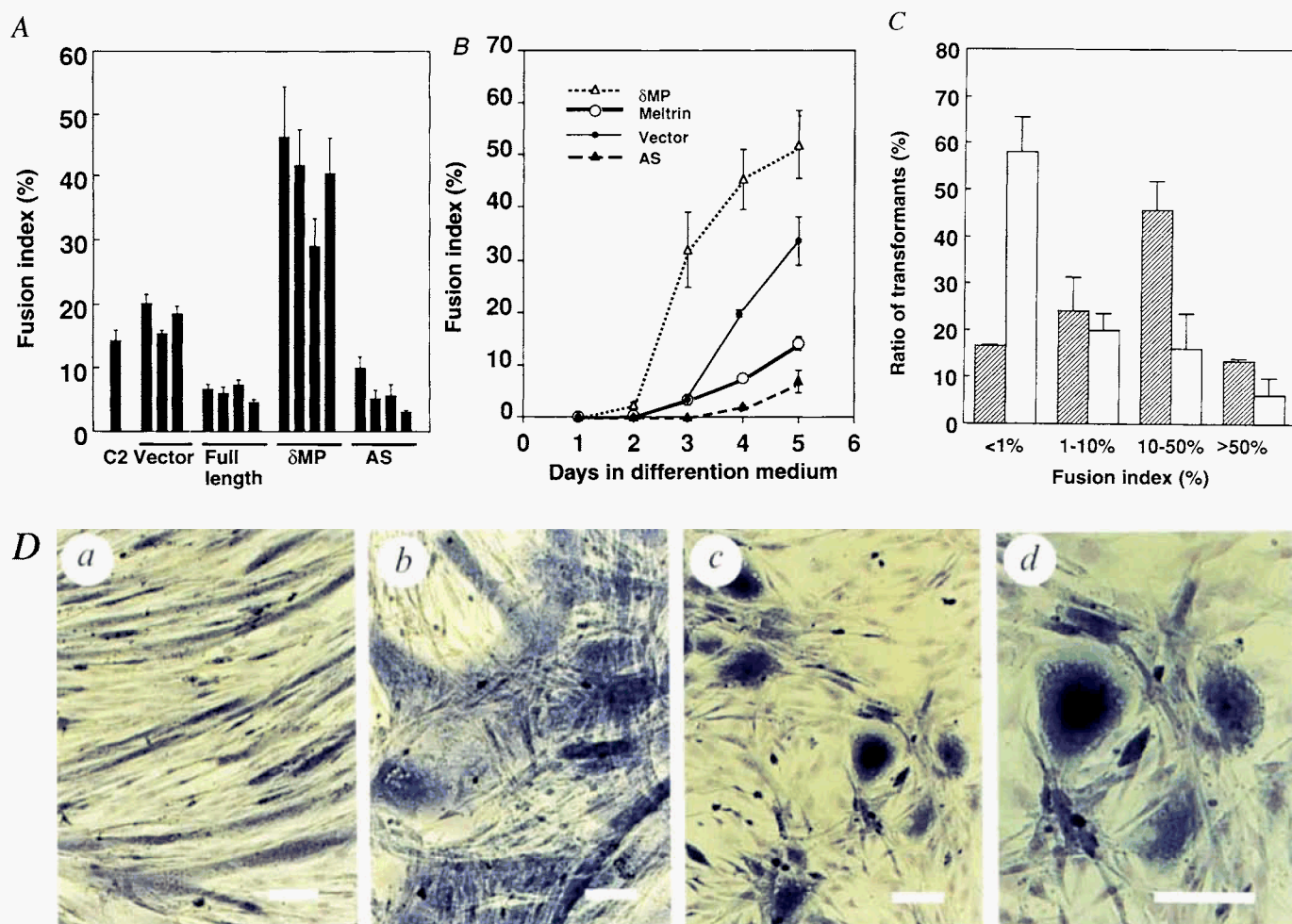


FIG. 4 Involvement of meltrin- α in myotube formation. **A**, **B**, Effects of the full-length, δ MP and the antisense construct (AS) for δ MP on fusion. Bars indicate standard error of the mean. In **A**, fusion indexes (ratio of nuclei in myotubes) of four independent transformants 4 days after induction of differentiation were shown for each construct. In **B**, increasing rates of fusion index of transformants were shown. **C**, Comparison between fusion capability of the AS- (white bars) and the vector-transfected (shaded bars) primary colonies. Approximately 100 transformants were categorized by fusion index. The result shows the average of two independent transfections. **D**, Typical myotubes of the vector- (**a**) and δ MP-transformants (**b** and **c**). **d**, Higher magnification of **c**. Expression of δ MP results in formation of more isotropic multi-nucleated cells with less tubular structures. Scale bars, 100 μ m.

METHODS. In **A** and **B**, clones were isolated in the existence of bFGF (5 ng ml⁻¹) to suppress myogenesis³⁰ after cotransfection of expression plasmids with pSV2NEO (at a molar ratio of 20:1). Expressions of various forms of meltrin- α in the full-length-, δ MP- and AS-transformants were 50–150, 10–50 and 0.2–0.3-fold of that in parental C2, respectively. Transformants were seeded at 2×10^5 per plastic dish of 6 cm diameter 1 day before induction of differentiation. To determine the fusion index, four independent fields of 1 mm² were counted after fixation. There was no significant difference in growth rate among transformants with different constructs (data not shown). In **C**, the cells were seeded to get approximately 50 G-418 resistant clones per plastic dish of 9 cm diameter after transfection. Clones grown for 10 days were induced to differentiate for 2 days before fixation. Fusion index was counted in a field of 1 mm² at the centre of each colony.

prove to be important in cell-cell or cell-matrix interactions during development. □

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Plasmid-based transgenic mouse model for studying *in vivo* mutations

Michaël E. T. I. Boerrigter, Martijn E.T. Dollé, Hans-Jörg Martus, Jan A. Gossen & Jan Vijg

A new transgenic mouse model for studying *in vivo* somatic mutations is based on the efficient recovery of chromosomally integrated *lacZ*-containing plasmids, using magnetic beads.

THE analysis of mutations in various organs and tissues of higher animals is important to obtaining more insight into the mechanisms of *in vivo* mutagenesis and for evaluating the mutagenic and carcinogenic potential of chemical and physical agents. A major difficulty in studying *in vivo* somatic mutations in chromosomal DNA of higher organisms has been the lack of techniques capable of identifying and isolating mutated genes with high efficiency. Several methodologies for detecting *in vivo* somatic mutations are available, but most of these methods rely on propagation of cells *ex vivo*, are cumbersome and/or have a very limited sensitivity to different kinds of mutations^{1,2}.

Thus far, the only methodology for the detection and characterization of mutations in different organs and tissues is based on the use of transgenic mouse models harbouring chromosomally integrated bacteriophage- λ shuttle vectors, equipped with bacterial reporter genes. The first such mouse model, using the bacterial *lacZ* gene as a target for mutagenesis, was developed in our laboratory³. The other model, developed by Short and co-workers, employs the *lacI* gene as mutational target^{1,4}. Both models are presently under evaluation⁵.

Bacteriophage- λ models

A major limitation of bacteriophage- λ shuttle vectors is that an important class of mutagens, termed clastogens, has been found to yield very low responses^{6,7}. On the assumption that clastogens cause predominantly (large) deletions, the low response rate might be explained as being due to the difficulty of packaging λ vectors smaller than 42 kb or larger than 52 kb. In addition, deletions extending into regions adjacent to the transgene concatemer are not detected, as two intact *cos*-sites are required for the packaging of a single λ vector. Indeed, in the bacteriophage- λ systems large deletions have never been found. Most deletions that have been reported are very small, that is, between 1 and 23 base pairs, and deletions in the range of hundreds of base pairs were only reported once or twice⁸. In this respect

it can be hypothesized that the tandemly integrated bacteriophage- λ cluster, representing a total of about two million base pairs of prokaryotic DNA in these models, is not the optimal substrate for the mammalian DNA-processing enzymes generating rearrangements.

Large structural alterations are a considerable fraction of the spontaneous mutation spectrum in one of the few selectable target genes that can be analysed in human cells, that is, the X-linked hypoxanthine phosphoribosyl transferase (*HPRT*) locus². Mutations

bacteriophage- λ models is the relatively low recovery of these vectors from genomic DNA. Indeed, intactness of the genomic DNA is critical in obtaining maximum efficiencies in transgene rescue. However, in view of the large size of the bacteriophage vectors the occurrence of large numbers of DNA breaks during DNA extraction is unavoidable, with apparent negative consequences for the rescue efficiencies. In practice, between 10,000 and 25,000 plaque-forming units (PFU) are obtained per microgram of total genomic DNA^{9,10}, which is about 3 per cent of the maximum efficiency possible (assuming a copy number of 40 and a packaging efficiency of 10⁹). To overcome all the restrictions associated with the current models, we designed a plasmid-based transgenic animal model and developed the methodology to rescue plasmids from their integrated state into *Escherichia coli* hosts¹¹. By using a positive selection system, only plasmids harbouring a mutant *lacZ* gene give rise to a colony. These principles have now been worked out in a practical assay applicable to a C57Bl/6 transgenic mouse, homozygous for the pUR288 plasmid. The *lacZ* reporter transgene is part of the pUR288 plasmid, of which approximately 20 copies are chromosomally integrated head to tail.

Plasmid rescue

Figure 1 shows the integrated plasmids and the procedures used to separate them from the mouse genomic DNA after restriction enzyme digestion and their subsequent electrotransfer into *E. coli* to inspect the reporter gene for mutations. Since plasmid rescue is not as size-dependent as bacteriophage- λ

in vitro packaging, even large deletions extending into the flanking regions of the host chromosomal DNA should be detectable, provided the antibiotic resistance gene and the origin of replication are recovered. Hence, besides point mutations, all kinds of DNA rearrangements are detectable. In Fig. 1, this is illustrated by the rescue of a large deletion, extending into the 3'-flanking region of the transgene cluster. It could be

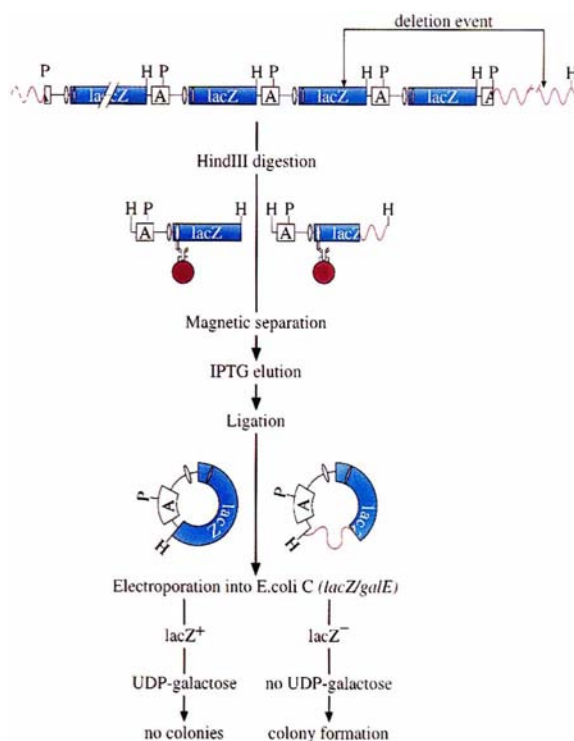


FIG. 1 Rescue of *lacZ* pUR288 plasmids integrated head-to-tail in the mouse genome.

in *HPRT* can only be measured in cells that can still actively proliferate *in vitro*, such as human T lymphocytes. As a mutational target gene, *HPRT* may therefore not be representative of the situation in other cell types. Nevertheless, it seems likely that the mutation spectra obtained with the current bacteriophage- λ models do not reflect the true *in vivo* mutation spectra.

A technical problem associated with

predicted that this will necessarily result in the inclusion of a mouse genomic DNA fragment into the rescued plasmid.

Plasmids containing the *lacZ* reporter gene are excised from the genomic DNA by *Hind*III restriction enzyme digestion and separated from total genomic DNA using *lacI* repressor proteins coupled to magnetic beads¹¹. After circularization by ligation, the plasmids are electrotransferred into *E. coli* *C lacZ⁻, galE⁻* host cells. The bacteria are host-restriction-negative, which prevents degradation of incoming (methylated) plasmid DNA¹². The mutation in *galE* facilitates the positive selection of *lacZ* mutants on medium containing the lactose-analogue P-gal. *GalE* mutants lyse in the presence of galactose because they lack the enzyme UDPgal 4-epimerase¹³. Therefore, in this system the non-mutants are killed and only the mutant *lacZ⁺* cells can form colonies¹⁴.

It was found that the procedure is so efficient that in a single experiment millions of plasmid copies can be recovered. Per microgram of genomic DNA of about 100,000 transformants are now routinely obtained, which is about 30 per cent of the theoretical maximum efficiency (assuming a copy number of 20 and a transformation efficiency of 10^{10}).

Mutant frequencies

With the protocol described above, mutant frequencies were determined in different organs and tissues of untreated animals and animals treated with different mutagenic agents. The spontaneous mutant frequency in this model is between 4 and 7×10^{-5} as shown in Table 1. This falls in the same range as the values reported for the bacteriophage- λ models, although several authors have reported lower values⁸. A somewhat higher spontaneous mutant frequency with the plasmid model would not be surprising in view of its greater sensitivity for DNA rearrangement events. Indeed, in about half of the spontaneous mutants the *lacZ* gene appeared to have undergone size changes, mostly deletions varying from about 50 to 3,000 base pairs. As predicted and illustrated in Fig. 1, a few per cent of these size changes were found to contain mouse sequences. This was indicated by a positive hybridization signal with a [³²P]-labelled (non-transgenic) mouse genomic DNA probe^{15,16}.

Naturally, the question would arise as to whether all the background mutations detected found their origin in the mouse or could be artefacts of the rescue procedure. We identified at least three potential sources of artefacts. First, we studied the contribution of reversion of galactose sensitivity of the *galE⁻* strain. Reverse mutations in *galE* as well as forward mutations in *galT* or *galk¹⁴* would allow non-mutant cells also to grow on the selective medium. These are easy to identify because they are *lacZ⁺* and galactose resistant. We found the frequency of such false-positives to be about 2×10^{-6} and therefore quite negligible. Then, the electroporation

process could induce damage in the plasmid which could lead to mutations generated in *E. coli* during the first rounds of replication. This was checked by the electrotransfer and subsequent inspection for mutations of pUR288 plasmid, not prepared from the mouse, but in *E. coli* itself. A mutation frequency of less than 1×10^{-5} was found, which indicates a 10–20 per cent contribution of *E. coli* to the background mutant frequency. Most of these mutations, however, probably originate during plasmid growth in *E. coli* rather than after electrotransformation¹⁶.

Finally, the possibility had to be taken into account that the presence of the *Hind*III site so close to the *lacZ* mutational target gene could be responsible for at least a part of the deletion mutations. This was checked by using the *Pst*I site rather than the *Hind*III site in the plasmid rescue procedure (Fig. 1). The use of this restriction site, which is located in the ampicillin resistance gene, allowed us to select against ligation artefacts. The use of *Pst*I in the rescue procedure resulted in 10–30 per cent lower mutant frequencies. However, the use of *Pst*I rather than *Hind*III makes it impossible to detect large deletions involving mouse sequences flanking the integrated plasmid cluster. Indeed, mouse sequences were found in 1–20 per cent of the deletion mutants after *Hind*III digestion (depending on the tissue or treatment) they were never found after *Pst*I digestion (or, for that matter, after mixing plasmids grown in *E. coli* with non-transgenic mouse DNA followed by *Hind*III digestion). In addition, virtually all deletion mutants including the ones contain-

ing mouse sequences were found to contain only one *Hind*III digestion site without any evidence for a predominance of deletions close to or over this site. From the above we conclude that at least most of the background mutants must have originated in the mouse. However, a more careful examination of the mutational spectra is necessary before any definite conclusions can be drawn.

The capacity of the system to detect induced mutations is illustrated by an elevated mutant frequency after treatment with different known mutagenic agents. Several organs were analysed, but in Table 1 only the results for the spleen are compared. Whole-body irradiation with 50 rads of X-rays on five consecutive days resulted in an approximately fourfold induction of mutations, with about the same percentage of deletion mutations as in the untreated animals. An intraperitoneal injection of 250 mg per kg body weight of ethyl nitrosourea (ENU) resulted in a 17-fold

Applications

Efficient plasmid purification, together with positive selection of mutant plasmids, make the pUR288-C57Bl/6 transgenic mouse a powerful model for studying a broad spectrum of mutations in a variety of cell types *in vivo*. The model should be useful for directly studying a number of hypotheses linking somatic mutations to physiological and pathological end points, such as cancer and aging. In addition, the effect of individual genes involved in DNA damage metabolism (for example, antioxidant defense, DNA repair) on mutation frequency and spectra can be studied directly in double transgenics, that is, in the plasmid mouse in which specific genes have been altered or inactivated.

A more practical application involves genetic toxicology testing. Strategies for evaluating mutagenic hazards *in vivo* are lacking suitable endogenous genes for studying mutations in animal tissues. The present model system, which is sensitive to a broad spectrum of mutations including large structural alter-

tations, would fill in this gap in a cost-effective manner. A key issue here is whether or not a surrogate target gene provides a realistic picture of the actual situation in the genome. However, in view of the large variety in genomic environments, there is probably also no single natural gene or genomic region that reliably reflects the situation overall.

In the practical application of the plasmid model in studying somatic mutagenesis, three factors are of major importance. First, the plasmid vector is much smaller than bacteriophage- λ , which greatly simplifies DNA extraction. Indeed, rapid (automated) DNA extraction procedures can easily be used and still provide very high vector rescue efficiencies. Second, the efficiency of plasmid rescue is very high and therefore permits highly accurate determinations (based on large numbers of colony-forming units) or determinations on small amounts of tissue. Third, the possibility of detecting *lacZ* mutants by a

TABLE 1 *LacZ* pUR288 plasmid mutant frequencies in spleen

Treatment	Dose	Time	MF ($\times 10^{-5}$)	n	Mutation type (%)		Inserted mouse size-sequences (%)
					no change	size change	
Untreated	—	—	5.8 ± 0.6	6	54	46	7.6
ENU	250 mg kg ⁻¹	14 d	97 ± 19	3	86	14	1.4
X-rays	5×50 rads	10 d	21.3 ± 2.9	3	53	47	4.7
B[a]P	100 mg kg ⁻¹	14 d	20.1 ± 3.7	3	70	30	2.0

simple selection procedure, rather than on the basis of colour, greatly simplifies the assay. These three features should reduce the costs of mutation frequency determination and permit large-scale *in vivo* mutation analysis studies. Indeed, the cost reduction as compared with the *lacI* and *lacZ* bacteriophage- λ models is about a factor of 10 and 5, respectively. The difference is explained by the fact that the positive selection system can also be applied to the *lacZ*, but not to the *lacI*, bacteriophage- λ model. These practical considerations, in combination with the broader sensitivity of the model to all kinds of mutational events lead us to predict that plasmid-based transgenic mice will be instrumental in obtaining insight in mechanisms of mutation induction in higher animals. □

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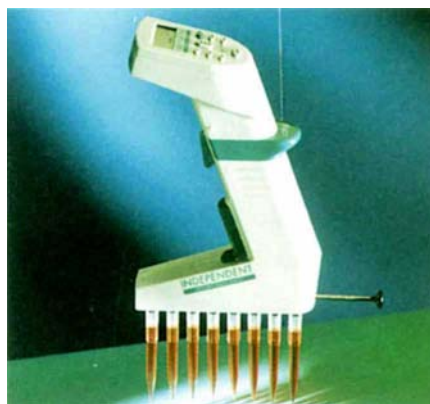
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Gene cipher

This week's issue offers items for cracking the genetic code, such as labelling reagents, transgenic laboratory products, an on-line molecular biology computing resource, a mammalian vector system, and more.

A NEW system, designed to simplify and speed the process of **RNA purification**, is now available from Hybaid (*Reader Service No. 101*). The Ribolyser RNA extraction system uses two features to generate pure RNA in approximately 30 min. The first is an instrument that uses high mechanical forces to quickly disrupt tissues and cells, the second, reagents that stabilize RNA and prevent degradation by nucleases. By utilizing a simultaneous shaking/twisting motion at high speeds, this instrument provides the conditions for cell disruption that are said to ensure a high yield of RNA. Flexibility is conferred by adjustable operating time and speed, and a rotor capacity of up to 12 samples allows multiple processing. To complement the instrument, a range of optimized reagent kits is also available for yeast, bacteria, algae and fungi, as well as for plant and animal tissues.

Integra Biosciences has announced the release of new **DNA and cell culture equipment**, including the Independent multichannel stepper pipette for cordless, electronic pipetting (*Reader Service No. 102*). Other new products include the Pipetboy with colour coding to keep pipettors identified for specific purposes. The company also offers Matrix disposable filter tips for PCR and other applications. For cell cultureware, there is also Cellspin and Cellroll, two products that are designed for flexible cell-mixing routines. These products can be adapted for large-scale cultivation and are said to be useful for problematic cell lines. The Tecnomat Petri dish pouring system is designed to complement the AgarClav, a combined autoclave and bulk media preparation unit. Finally, there is Tecnomouse, a bioreactor system that produces monoclonal



Integra Biosciences' Independent pipette.

antibodies without the need for animals. The manufacturer states that it consistently produces monoclonal antibodies from widely differing cell lines, including human, mouse and rat hybridomas, as well as CHO cells in concentrations up to 10 mg ml⁻¹.

A **purification system for plasmids** up to 300 kb in size for positional cloning or cytogenetic phenotyping is now available from the Nest Group (*Reader Service No. 103*). The Nucleobond AX ion-exchange cartridges are designed so that even the largest plasmids, BACs and PACs can be purified in high yield. Using a larger piece of DNA reduces both time and cost when doing chromosome mapping for cytogenetic phenotyping or positional cloning. These ready-to-use cartridges are supplied in kits that contain all of the required buffers, reagents and protocols. The procedure should prove useful for performing further manipulations including restriction analysis, subcloning, amplification and sequencing.

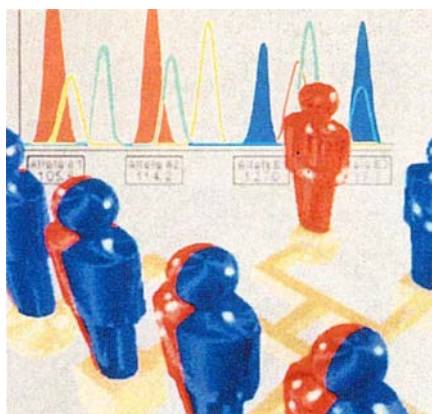
Lambda Fluoreszenztechnologie now offers **chromogenic and fluorogenic enzyme substrates** for the photometric and fluorogenic assay of hydrolases such as esterases, lipases, phosphatases, sulphatases, glycosidases and proteases (*Reader Service No. 104*). Aside from various traditional substrates, such as X-gal and 4-Mu substrates, new sophisticated hydrolase substrates are available from the company. A low pKa dye allows the direct and continuous kinetic assay of enzymes possessing a pH optimum.

RPMS Technology is currently seeking licensees to exploit a new **mammalian vector system** — designed to offer stable transfer of biologically functional information to a range of mammalian cells (*Reader Service No. 105*). The manufacturer states that this means of exogenous gene transfer to human cells has been developed with the use of pseudocapsids formed from the major capsid protein of polyoma virus. The pseudocapsids contain no viral genes and may prove to be less hazardous than viral vectors. Pseudocapsids appear to be capable of integrating relatively large fragments of exogenous DNA into the host genome and to give long-term stable expression. The particles may also be able to deliver antisense sequences, drugs, toxins or proteins such as antibody fragments.

Software

The Genotyper software from Perkin-Elmer's Applied Biosystems Division is a new addition to the company's automated **genotyping system**, which includes the model 373 DNA sequencer, the model 672 Genescan software and fluorescent, four-colour labelling reagents (*Reader Service No. 106*). This soft-

PRODUCT REVIEW



Perkin-Elmer's Genotyper software.

ware package takes PCR-based DNA fragment data (PCR product size and quantity) generated by the automated genotyping system and transforms it into genotype results automatically. The program is designed to quickly analyse large amounts of data by automatically identifying scoring alleles, equating peak ratios and determining peak statistics. The error identification features, such as the mendelian inheritance checker, help locate genotyping errors or misinheritance. It also features user-definable templates for customized analysis of DNA sizing, quantitation and pattern comparison of data. Sizing applications include automatic

genotyping of microsatellite-repeat markers for linkage mapping and genetic disease status, paternity or forensic identification.

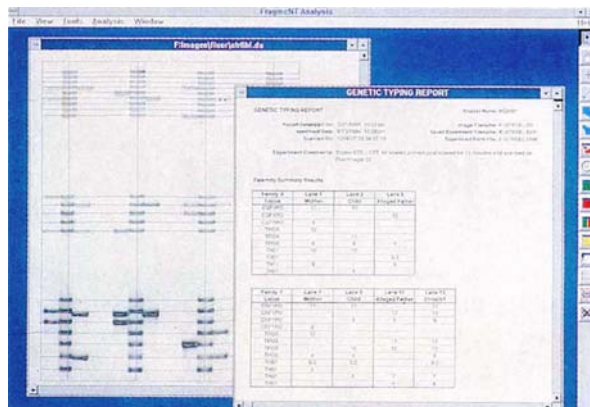
American Type Culture Collection in partnership with InforMax has developed Vector NTI to be a knowledge-based **software package, designed to automate many cloning applications** (Reader Service No. 107). It is said to be capable of designing new genetic molecules automatically and according to user specifications. The software contains over 3,000 rules for genetic engineering and 80 commonly used vectors, including those available for transferring changes to molecules in child-parent trees and visualisation of those changes. Additional features include a more powerful, user-friendly sequencer, and the ability to choose any number of options for new molecule design. All molecules can be analysed to identify sequences for PCR primers, restriction sites, open reading frames and sequence motifs.

Molecular Dynamics has developed **genetic typing software**, which is designed to provide automated assign-

ment of allele names to DNA data (Reader Service No. 108). The software comes with precoded RFLP markers and alleles for human short-tandem repeat reagents. The program has been designed for linkage, forensic and paternity testing laboratories and generates finished summary reports from several selectable report styles. Reports consist of quantitative information for each band and statistics related to deviations from expected results.

Informatics

A new range of **high-quality biochemicals** is now being offered by Promega (Reader Service No. 109). Biochemicals for molecular



Molecular Dynamics' genetic typing software.

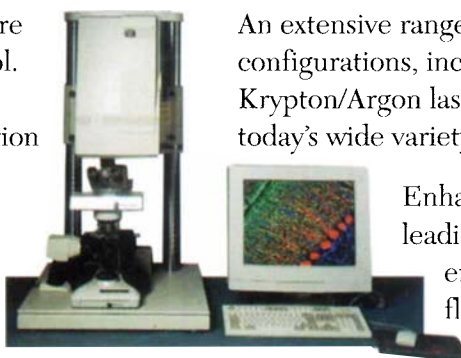
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biology, sequencing, detection and electrophoresis are included and are said to be use-tested. All molecular biology grade reagents are tested for the absence of DNase, RNase and protease contamination. The company can provide next-day delivery of all products with safety data sheets supplied with hazardous goods and certificates of analysis accompanying them.

IntelliGenetics, now offers its **molecular biology computing resources** through the company's Bionet on-line service via the Internet (*Reader Service No. 110*). The company states that the system offers up-to-date sequence data, comprehensive sequence analysis software, and high-performance searching algorithms that allow scientists to conduct bioinformatics research at an affordable price. These tools and resources are all contained within Bionet and maintained by IntelliGenetics. Users need only a computer with a modem or Internet access — the Bionet account includes expert, toll-free customer support for assistance in all phases of on-line research. Access is provided to proprietary databases as well as major sequence data banks, including GenBank, EMBL, PIR and Swiss-Prot.

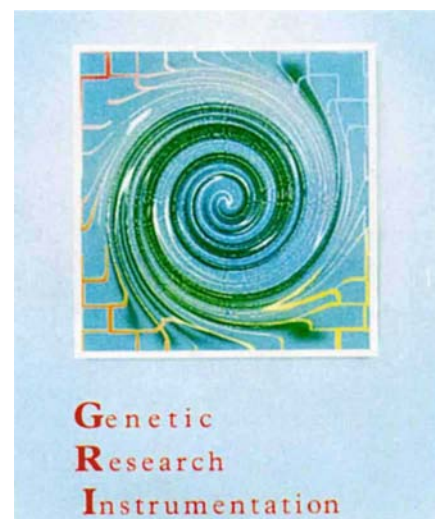
Derwent's Geneseq **database** contains information on nucleic acid and protein sequences

from patent applications and granted patents from 40 patent-issuing authorities (*Reader Service No. 111*). This database currently contains 110,000 sequences, and covers all patents issued from 1981 to the present. The database is both a source of information and a research tool for the scientist. Available with Geneseq on the STN network in December of 1995 is the *Patents Citation Index*, which is said to contain every patent from 16 patent-issuing authorities encompassing 15 million citations.

Catalogues

The 1995-96 Harlan Sera-Lab **catalogue** is now available (*Reader Service No. 112*). It covers monoclonal antibody production, hybridoma development, murine antibody-production testing, custom conjugation, genetic monitoring and antibody purification, as well as custom immunological services. The product line includes monoclonal and polyclonal antibodies, cytokines and growth factors, fetal calf and other sera, chromogens for western blots and ELISA assays, as well as organs, glands and tissues.

Genetic Research Instrumentation has just published their new catalogue that brings together **molecular biology research products** from a range of specialist manufacturers (*Reader Service No. 113*). Products, such as



Genetic Research Instruments catalogue.

handmade electrophoresis equipment from Atto, include the AE6200 for vertical polyacrylamide gel electrophoresis, which has the buffer chamber positioned behind and in contact with the full face of the gel plate. Also available from the company are the programmable thermal cyclers from MJ Research, including the PTC-225 four-headed thermal cycler. It has the capacity to be fully integrated into a robotic laboratory and will be

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PRODUCT REVIEW

available later this year. The catalogue also includes the full range of Labconco products, as well as freeze dryers, water purification systems, biological safety cabinets and centrifugal evaporation systems.

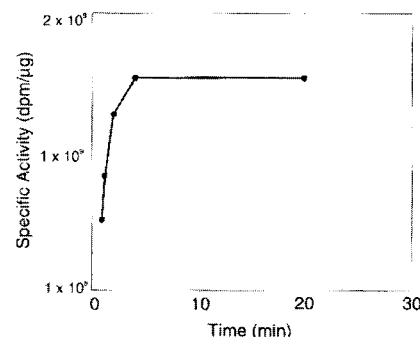
Probes and primers

Genosys Biotechnologies has introduced DecamerArray — a library of 1,040 ten-nucleotide oligomers useful for arbitrary priming applications, such as RT-PCR, fingerprinting and mapping techniques (*Reader Service No. 114*). The library is available in eleven 96-well plates, and also includes a PC diskette and a printed index containing sequences, plate locations and other pertinent information. The oligonucleotides are composed of differing GC content, with a distribution of oligonucleotide parallels, all 1,048,576 possible sequences have a GC content ≥ 50 per cent. The sequences have been chosen to represent the entire spectrum of diversity available after the elimination of palindromes, hairpins and self-dimerization.

Oncor offers two new **DNA probes for fluorescence *in situ* hybridization** (FISH) studies of acute myelomonocytic leukaemia (AML-M4), acute myeloid leukaemia and myelodysplastic syndrome (*Reader Service No. 115*). The 5q31-specific probe identifies deletions in chromosome band 5q31 and is particularly useful in situations where karyotyping fails to

identify the deletion. This probe can also be used in translocation analysis when chromosome 5q is involved and as a marker for gene mapping by FISH. The inv(16)(p13;q22) is a characteristic rearrangement seen in AML-M4, a subgroup sometimes associated with abnormal eosinophils, and myelodysplastic syndromes. The probe identifies the 16p13 breakpoint associated with inv(16)(p13;q22) in AML-M4.

Stratagene's Prime-It II **random primer labelling kit** produces high specific activity, DNA hybridization probes (1×10^9 integrations min^{-1} per mg^{-1}) in only two minutes (*Reader Service No. 116*). These probes should prove useful for Southern and northern analyses and screening gene libraries. The kit uses a genetically engineered 3' exonuclease-deficient clone of the Klenow fragment of DNA polymerase I, as well as random 9-oligomer primers. It can produce probes from fragments purified by a variety of techniques, including isolation from low-melting temperature agarose. It has also been designed to perform equally well with [^{32}P]dATP or [^{32}P]dCTP. The Prime-It Rm T kit has been developed to allow storage at room temperature by utilizing a heat-stable polymerase. A fluorescence labelling kit is also available, which allows the probe to be viewed directly using a fluorescence microscope.



The Prime-It II random primer labelling kit.

Taconic Farms has obtained the Genpharm **transgenic laboratory products** division (*Reader Service No. 117*). Taconic now offers all eight transgenic animal models including the TSG-p53 transgenic mouse. The company now produces more than 100 transgenic lines for customers under contractual agreements. The integration of production colonies, breeding programmes, genetic testing and the establishment of a new transgenic repository is said to offer researchers a comprehensive transgenic technology resource. □

These notes are compiled by Brendan Horton from information provided by the manufacturers. For more details, fill in the reader service card bound inside the journal.

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